Oral presentations

New aspects of emerging *Clostridium difficile* infections (CDI)

S2 Pros and cons of molecular fingerprinting for *Clostridium difficile* infections

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Since the 1980s the epidemiology of *Clostridium difficile* infection (CDI) has been investigated by the application of many different typing or fingerprinting methods. To study the epidemiology of CDI, a typing method with a high discriminatory power, typeability, stability, power, reproducibility and epidemiological concordance is required. It should also have technical advantages, such as ease of performance, relative low cost, and high throughput. A growing number of molecular methods have been applied to *C. difficile*. For the early and rapid detection of outbreak situations, methods such as restriction enzyme analysis, arbitrary primed polymerase chain reaction (PCR), pulsed-field gel electrophoresis, and PCR ribotyping are commonly used. For long-term epidemiology, multilocus sequence typing, multilocus variable number of tandem repeats analysis (MLVA), and amplified fragment length polymorphism are of interest.
Currently, the PCR-ribotyping method and the library of PCR ribotypes in Cardiff are the benchmarks to which most typing studies around the world are compared. Conventional agarose gel-based PCR ribotyping is easy to use and relatively cheap, but analysis of fragment lengths is hampered by poor resolution. Recently, a capillary gel electrophoresis-based PCR ribotyping assay has been developed that significantly reduces the hands-on time required for *C. difficile* PCR ribotyping. The results were highly reproducible, independent of reagent batches or brands used and allows inter-laboratory comparisons of typing results.
The analysis of the sequenced *C. difficile* genome revealed a high percentage of DNA that consisted of a variable number of tandem repeats (VNTR). Recently, a new MLVA method was developed using smal short tandem repeats (2–9 bp) to facilitate automated fragment analysis with multicoloured capillary electrophoresis instead of sequencing. In a study using isolates from laboratories in Canada, the Netherlands, the United Kingdom, and the United States, seven *C. difficile* typing techniques were compared, but only REA and MLVA showed sufficient discrimination to distinguish strains from different outbreaks. MLVA has also been applied to study local outbreaks of *C. difficile* PCR ribotype 027 strains. MLVA is currently the most discriminative typing method and will contribute significantly to our understanding of the epidemiology of *C. difficile*.

S3 New treatment aspects of CDI

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The need for new therapeutic alternatives in CDI is clear. Current recurrence rates are unacceptably high. The optimal treatment of severe CDI, infection caused by strains associated with poor outcome (e.g. *C. difficile* ribotype 027), and multiple recurrences is unclear. There are several potentially promising new CDI treatment approaches under investigation, including antibiotic, anti-toxin and bacterial interference options. Probiotic therapy remains of unproven benefit. As new options become available, prognostic data will be needed to guide on the most approriate therapeutic choices.

S4 Emerging community-acquired CDI in North America and Europe

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Clostridium difficile has long been recognised as important cause of antibiotic associated diarrhoea in hospitalised patients. Although community associated disease has been described in the 80's, it is only in this decade that it has been recognised as an important cause of infectious diarrhoea in patients in the community.
As *C. difficile* was mostly recognised as a nosocomial pathogen, the majority of studies of clinical *C. difficile* infections (CDI) have been conducted in acute care hospitals, and many have been performed during outbreak situations. The hospital environment has very specific characteristics which are likely to be important determinants of infectious disease occurrence and transmission, such as clustering of susceptible hosts, increased possibility of environmental contamination, physical proximity and multiple person to person contacts. Similarly, the demographics of hospitalized patients population also differ significantly from that of the community. In hospitals, the prevalence of antibiotic use is very high, and highly correlated with many other factors which could be important in the expression of CDI. Until recently antibiotics were believed to be a prerequisite for the development of CDI but studies of community acquired disease are consistently describing disease occurring in a high proportion of patients who have no such exposure. This has lead to research examining other risk factors.
CDI appears to be increasing in the community especially in the elderly, but also in the pediatric population. As up to 50% of CDI in the community may not be precceded by recent antibiotic exposure particularly in patients without a recent hospitalization, many of the recommended diarrhea testing alogarithms will result in this diagnosis being missed or delayed. This could have important implications for patient care and control of outbreaks. Well designed studies of *C. difficile* in the community could improve our understanding of this disease and improve the ability to explore other risk factors and where antibiotic exposure maybe less confounded.

Presentation of International Sepsis Forum Award

S5 Genetic analysis of pattern recognition receptors links a functional polymorphism in the gene for CD14 to susceptibility for meningococcal septic shock

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Objectives: Contact with, and colonization by, pathogenic meningococci occurs rather often. Still, only a limited number of individuals develop invasive disease, most often within a few days after acquisition of a new meningococcal strain. Thus, it can be assumed that disease develops because of defects in the early innate defense. We hypothesized that polymorphisms in Pattern Recognition Receptors (PRRs) influence susceptibility to meningococcal disease or alter disease severity.
Methods: 73 different single-nucleotide polymorphisms (SNPs) in 21 genes encoding PRRs and related molecules of the innate immune system were investigated using a research prototype of a line-probe assay (INNO-LiPA, Innogenetics). We performed a three-stage approach. First (stage 1) we performed a case-parent study in subjects with meningcococcal disease admitted to the intensive care unit (ICU) (n = 118). Second (stage 2), we performed a case–control study in an

extended cohort (n = 147). Finally (stage 3), the observed associations were tested for replication in an independent case–control study (n = 146).

Results: In stage 1, we found SNPs in the genes for MASP2, CD14, LBP and TLR6 to be associated with increased susceptibility to meningococcal disease. For the rs2563298 SNP in CD14 this was replicated in the case control study (stage 2; OR 1.65, 95% CI 1.17–2.31) and in the separate replication study with the independent (confirmatory) cohort (Stage 3; OR 1.44, 95% CI 1.02–2.04). Subgroup analysis showed that the CD14 SNP conferred increased risk only in shock patients (OR 1.99, 95% CI 1.28–3.08). This CD14 SNP is functional and leads to decreased IL-10 and IL-12 production in a whole blood model of meningococcal sepsis.

Conclusions: In conclusion, we identified a functional SNP in CD14 that is associated with an increased risk for meningococcal septic shock. Possible other SNPs associated with increased risk for meningococcal disease were in MASP2, LBP and TLR6.

S6 Histones in sepsis

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Chromatin is made-up of DNA, histones and non-histones proteins. Covalent modifications of histones through acetylation at Lysine residues strongly influence the structure and the function of the chromatin. Whereas acetylation of histones results in a relaxed chromatin structure that is associated with active transcription, de-acetylation of histones results in a compacted chromatin structure associated with repressed transcription. Global histone acetylation is regulated by the opposing actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Beside histones, non histones proteins are modified by reversible acetylation, among which a-tubulin, HSP90 and transcription regulators. Therefore, HDACs impact on many biological functions, primarily cell differentiation, growth and survival. HDAC inhibitors (HDIs) were originally developed for their powerful anti-cancer activity. Yet, recent preclinical studies suggest that HDIs possess anti-inflammatory activity. Based on these observations, we postulated that HDIs could impact on innate immune response to microbial infection.

Here we will discuss the results from our studies on the effect of HDIs on the innate immune system. We first performed genome-wide gene expression analyses to have a global view of the impact of HDIs on the transcriptome of resting and microbial product-stimulated primary macrophages. We then studied the influence of HDIs on key parameters (activation of the intracellular signal transduction pathways, production of cytokines and chemokines, expression of co-stimulatory and chemokine receptors..) of macrophages, dendritic cells and whole blood activated by a broad range of microbial products. Finally, we analyzed the impact of HDIs in preclinical models of non-severe bacterial and fungal infections, toxic shock and severe sepsis.

Overall, these studies demonstrate that HDIs are powerful anti-inflammatory and immunosuppressive drugs that impair innate immune responses to microbial infections *in vitro* and *in vivo*. While the results suggest that HDIs may represent attractive adjunctive therapies to treat pathological situations characterized by dysregulated inflammatory responses such as autoimmune diseases and severe sepsis, they also warn that HDIs may increase the risk of developing opportunistic infections and sepsis, especially in immunocompromised cancer patients.

Latest trends from the molecular laboratory for studying fungal pathogens and invasive fungal infections

S14 Diagnostic PCR for IFI. Does it really work?

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Invasive aspergillosis (IA) continues to have high mortality, partly as a consequence of the difficulties of early diagnosis and also due to therapeutic limitations. Nowadays IA diagnosis is based on extensive use of galactomannan (GM) and high-resolution chest tomography (HRCT). Despite the fact positive GM has prompted an earlier treatment of IA, mortality is still high in this population showing that other diagnostic approaches should be taken into account. Detection of nucleic acids, PCR-based methods mainly, appears to be an option to assess although to date there are many doubts as to the profitability of this type of methods.

Diagnostic PCR must be considered an additional test that is being developed and that is used in some reference laboratories. Its lack of standardization should be stressed, since different laboratories use different approaches in terms of extraction, probes, primers, PCR conditions, and measurement. Furthermore the benefit of detecting DNA of *Aspergillus fumigatus* depends on the clinical sample used. PCR techniques have shown high diagnostic reliability in tissue biopsies and respiratory samples with high negative predictive values and discreet positive predictive values, but lower diagnostic reliability has been obtained in blood samples. Those high negative predictive values could be useful to rule out the presence of infection.

Newer approaches such as serial determinations of *Aspergillus* DNA in serum or blood, and detection of fungal DNA in higher volumes of blood have shown a greater diagnostic applicability, which increases when combined with GM quantification and high-resolution chest tomography. Those approaches have had higher rates of both sensitivity and specificity and improved the early diagnosis of aspergillosis.

Fewer data are available for other mycoses. PCR methods have been developed to detect *Candida*, endemic mycosis and emerging pathogens but more data are needed and reliable conclusions should not be drawn.

S16 Clinically relevant novel fungal taxa from old species complexes

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Our understanding of causative agents of fungal diseases has changed considerably during recent years. This is mainly due to the large scale introduction of molecular studies, in which one or more stretches of DNA are compared across a wide range of fungi, including human and animal pathogens. One of the most striking discoveries was the recognition of *Pneumocystis* as a fungus, as this organism was considered previously to belong to the parasites. In many cases traditional 'species' turned out to represent species complexes and many of the newly recognized clinically relevant species may differ in the susceptibility to commonly used antifungals. Therefore, correct identification of these new pathogens is important for proper patient management.

A brief overview will be presented of these developments in fungal taxonomy and their implications for the clinical practice. Examples will be given from the main lineages of the fungal kingdom, namely Zygomycetes, Ascomycetes (*Aspergillus*, *Fusarium*, and *Candida* spp.) and Basidiomycetes (Malassezia, Trichosporon and *Cryptococcus* spp.). Due to our own experience with the basidiomycetous yeasts we will give more in depth information on these fungi. The number of recognized species is rapidly increasing due to the application of molecular systematics and the availability of a large database of ribosomal DNA (rDNA) sequences, most notable the D1/D2 domains of the LSU rDNA and the ITS 1 and 2 regions. Only a few species are generally recognized as important pathogens for humans and animals, including *Cryptococcus neoformans* and Cr. gattii, several Trichosporon species and Malassezia spp. However, several non-conventional basidiomycetous yeast species can cause infection or are otherwise involved in health problems, such as hypersensitivity pneumonitis. We will present data on the involvement of these emerging basidiomycetous species, such as *C. adeliensis, C. diffluens, C. flavescens* and *C. laurentii* in human diseases. Emphasis will be on the Cr. neoformans-Cr. gattii and Malassezia species complexes, especially the role of the newly recognized species in disease, virulence, and susceptibility to antifungals.

S17 Is molecular subtyping a clinical tool?

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The main application of genotyping is for the investigation of epidemics or tracking routes of contamination. Several molecular typing techniques have been developed for fungi. Ideally, typing results should be accurate, reproducible, and easy to interpret. Importantly, methods should be transferable to other settings. Many typing methods such as randomly amplified polymorphic DNA, restriction fragment length polymorphism, single-strand conformation polymorphism analysis, and amplified fragment length polymorphism yield fingerprint profiles that are difficult to reproduce in different settings. In contrast, two methods have emerged for clinical applications as providing reliable, portable and easy to obtain data: microsatellite markers and Multi Locus Sequence Typing (MLST).
Microsatellites, or short tandem repeats, are defined as tandemly repetitive stretches of two to five nucleotides. After amplification, PCR products are analyzed based on amplicon length. The present limitation of microsatellite typing is transferability. A straightforward and universally applicable method to achieve such a calibration is through the use of allelic ladders. MLST is based on sequencing several housekeeping genes. Active MLST schemes are publicly available (http://www.mlst.net/) for yeasts. The main advantage of MLST is the ability to provide indisputable data based on sequencing. However, MLST is laborious, based on the quality of the sequences, has a long turn-around time and is associated with significant costs.
The nosocomial acquisition of invasive infections has been investigated. The conclusion are disappointing for invasive aspergillosis but unambiguous for invasive candidiasis. However, genotyping itself is not sufficient. Sampling, collection of clinical and epidemiological data are compulsory to reach a meaningful conclusion. Another interesting point is the propagation of a given clone with a resistant phenotype. This has already been investigated for *Aspergillus fumigatus* and *Candida tropicalis*.
Getting more and more pieces of information on the genome of each microorganism will probably be part of the future routine laboratories. The current trend towards mass sequencing creates such opportunities. This technology might lead to several modifications in the way we identify a species or characterize an isolate.

Pulmonary hypertension and infectious diseases

S19 Pulmonary arterial hypertension related to HIV: update on diagnosis and treatment

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The introduction of the highly active antiretroviral therapy (HAART) has profoundly influenced the course of HIV infection, improving the survival of HIV infected patients and reducing HIV-associated opportunistic infections. Nevertheless, long-term outcomes secondary to HIV infection are now serious concerns, like non-infectious cardiovascular complications including cardiomegaly, pericarditis, myocarditis and pulmonary arterial hypertension.
The lung is the most frequent target organ for disorders associated with HIV infection, and the cardiopulmonary vascular system can be sometimes involved; indeed, HIV-related pulmonary arterial hypertension (HRPAH) affects more HIV-infected individuals (i.e. 0.5%) than uninfected (i.e., 1 to 2 cases per million people).
The average age of HRPAH patients is 33 years, although the range can span from infancy to old age. There is no trend between HRPAH and HIV viral loads or CD4+ T cell counts but is more severe in AIDS patients. Shortness of breath is the most common symptom, and a clinical, cardiological, radiological work up is required for diagnosis. Cardiac catheterisation is the gold standard for the final diagnosis, and is mandatory to characterize the disease and exclude secondary causes.
There is no definitive evidence of HIV as a causal agent for HRPAH. However, viral proteins and its interactions with molecular partners in the infected host are strong candidates for cause-effect relationships because they may promote apoptosis, growth and proliferation. At least three of the HIV proteins are implicated in the pathology of PAH: the HIV envelope glycoprotein-120 (Env), the HIV protein Tat and HIV Nef (negative factor). Nef impairs vasomotor functions in pulmonary artery cells, decreases the expression of endothelial nitric oxide synthase and increases oxidative stress, suggesting a strong role in the formation of plexiform lesions in the lung vasculature.
The role of antiretroviral therapy (ART) in HRPAH is still debated. From a literature review, survival rates are 69% and 38% among patients treated or not with ART and specific therapy for PAH, respectively ($p = 0.02$), thus suggesting that specific therapy for pulmonary arterial hypertension should be strongly recommended in these patients. The role of the combination treatment with antiretrovirals in influencing the outcome of HRPAH is controversial, even if some evidences seem to indicate a beneficial effect in the clinical course of the disease.

DNA-based methods to detect MSRA

O22 Comparison of different bacterial DNA isolation methods to accelerate differentiation of *Staphylococcus aureus* from coagulase negative staphylococci from blood culture material

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Objectives: This study aims to compare 6 different DNA extraction methods from 2 commonly used blood culture materials, BACTEC (BD) and Bact/ALERT (Biomerieux), to accelerate differentiation between *S. aureus* and Coagulase Negative Staphylococci (CNS).
Methods: Two fast real-time PCR duplex assays, targeting the Tuf gene, to differentiate *S. aureus* from CNS, were developed in order to select the most sensitive one. This Tuf RT-PCR was used to compare 6 different DNA isolation protocols on two different blood culture systems. Negative blood culture material was spiked with *S. aureus;* bacterial DNA was isolated with: automated extractor EasyMAG by using 3 different protocols (Biomerieux), automated extractor MagNA Pure (Roche), a manual kit MolYsis Plus (MolZyme), and a combination between MolYsis Plus and the EasyMAG. The most optimal isolation method was used to evaluate the possibility of reduced bacterial culture times.
Results: Approximately 160 positive blood cultures containing Gram-positive cocci in clusters were tested in the Tuf RT-PCR and all were identified correctly. Bacterial DNA isolation, from spiked blood culture material, with the MolYsis Plus kit in combination with the EasyMAG showed the highest analytical performance, with a detection limit of 10 CFU/ml in Bact/ALERT material, whereas using BACTEC resulted in a detection limit of 100 CFU/ml. With this sensitive bacterial DNA isolation method 1 CFU/ml *S. aureus* in Bact/ALERT material was detected after a 5 hour pre-culture step in the Bact/ALERT3D.
Conclusions: A sensitive RT-PCR was developed for detection and differentiation of *S. aureus* versus CNS. Bacterial DNA isolation with the MolYsis Plus kit in combination with the EasyMAG resulted in the most sensitive detection of *S. aureus,* with a detection limit of 10 CFU/ml, in Bact/ALERT material. An initial *S. aureus* load of 1 CFU/ml can be detected after 5 hours incubation in Bact/ALERT3D by usage of the TUF test.

O23 Comparison of MRSA detection by Xpert MRSA test, Xpert MRSA/SA nasal test and culture

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Objectives: PCR based tests are increasingly being used to screen patients for MRSA carriage. Most commercially available tests use the SCCmec / orfX junction as one of the main targets for amplification. However, false positive MRSA results have been described due to

the presence of incomplete SCCmec cassettes in meticillin sensitive *S. aureus* (MSSA). Recently, PCR tests have been developed that detect targets, in addition to SCCmec, to overcome the problems of associated with partial SSCmec. Our study compares the detection of MRSA by the Xpert MRSA test (single target) with the Xpert MRSA/SA nasal test (multiple targets) and a sensitive enrichment culture method.
Methods: Nasal samples were collected with double-headed swabs from patients admitted to the Sandwell and West Birmingham Hospitals NHS Trust. Swabs were tested for MRSA using the Xpert MRSA/SA nasal test and the Xpert MRSA test (Cepheid Europe) run on the Cepheid GeneXpert system. All samples were also cultured for MRSA and MSSA using 2.5% NaCl broth and sub-cultured onto chromogenic MRSA agar and non-selective blood agar.
Results: 304 nasal swabs were included in the study (189 Xpert MRSA positive; 115 Xpert MRSA negative). MRSA was cultured from 92/304 (30.3%) of the swabs. The Xpert MRSA /SA nasal test versus MRSA culture showed a sensitivity, specificity, PPV and NPV of 84.8, 91.0, 80.4 and 93.2, respectively. 23/48 MSSA (so far analysed) isolated from nasal swabs testing PCR positive, but culture negative were identified as presumptive SCCmec mutants (SCCmec/chromosome junction positive; mecA negative). All 23 isolates were positive by the Xpert MRSA test whilst only 6/23 isolates were MRSA positive by the Xpert MRSA/SA nasal test.
Conclusion: Both the Xpert MRSA and MRSA/SA nasal tests showed high NPVs compared with MRSA culture making them useful as screening tests for MRSA. However, the benefit of detecting multiple targets (SCCmec/orfX, mecA, spa) by the Xpert MRSA/SA nasal test compared with only SCCmec/orfX by the Xpert MRSA test was reflected in the reduced number of false positives reported using the Xpert MRSA/SA nasal test. Our sample group was skewed to contain large numbers of PCR positive samples to increase to likelihood of detecting false positives. In a normal sample population, false positive results would only constitute a very small proportion of the overall results.

O24 Rapid detection of MRSA in screening specimens during a hospital outbreak

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Objectives: To compare the results of rapid PCR screening for MRSA using the GeneXpert system with those of cultures in an outbreak setting.
Methods: GeneXpert was used for screening MRSA in nose, throat, groin, and other clinical samples during a 6-month period. Samples were performed using a double-swab transystem. When >1 sample was found positive in a screening set, all second swabs of the set were analysed by culture.
Results: From June to October 2009, 7568 rapid tests were performed, among which 432 (5.7%) were positive (nose: 149/2090, 7.1%; throat: 98/2078, 4.7%; groin: 152/2080, 7.3%; urine: 14/1090, 1.3%; wounds: 18/150, 12%; and others:1/27, 3.7%), and 84 (1.1%) were invalid. A total of 1517 samples were analyzed by both rapid PCR and culture. Rapid tests had a sensitivity of 0.896 compared to cultures, a specificity of 0.769, a PPV of 0.763, and a NPV of 0.899. The rapid test was found to be less sensitive in throat samples (0.81) than in nose or inguinal samples (0.93 for both). In 32/192 (16%) patients a positive rapid PCR result was not confirmed by culture, despite several subsequent screening samples in some patients. Cycle threshold (Ct) for SCCmec of these PCR positive reactions were all >30.
Conclusions: GeneXpert MRSA was found to be suitable for the rapid detection in nose, inguinal, and throat samples, however with a lower sensitivity in the later. Negative cultures in 16% of our PCR-positive patients raised the question of false positivity or higher sensitivity of GeneXpert. Further work is needed to investigate these cases.

O25 Performance of the novel NucliSens EasyQ® MRSA assay with a diverse selection of clonal complexes from a low prevalence country

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Objectives: Several commercial assays have been developed for the rapid detection of MRSA colonization directly from nasal swabs, but genetic variations within the SCCmec cassette poses a constant challenge in the primer design. Another pitfall is the sharing of the SCCmec cassette among *S. aureus* and coagulase-negative staphylococci. NucliSENS EasyQ® MRSA (bioMérieux) is a novel test which simultaneous detects both the mecA gene and a specific cassette junction confirming the presence of the SCCmec cassette integrated in the *S. aureus* chromosome. The objective is to evaluate the performance of the assay with a collection of community-acquired MRSA strains from a low prevalence country.
Method: MRSA isolates were obtained from residents in North Jutland, Denmark and were characterized genetically by spa typing and clustered into spa clonal complex (CC) groups (www.ridom.de) as part of the Danish national surveillance program maintained by Statens Serum Institut. Cultures of MRSA strains were processed according to the manufacturer's guidelines for positive controls and analysed on the EasyQ instrument, a NASBA based platform.
Results: The study comprised 15 MRSA strains representing 10 different clonal complexes [number in brackets]: CC1 [1], 5 [3], 8 [1], 22 [2], 30 [1], 59 [1], 72 [1], 88 [3], 97 [1], 398 [1]). Eleven were reported to be MRSA whereas 4 were found MRSA negative (CC5 [1], CC59 [1], CC97 [1], CC398 [1]). The mecA gene was reported for all 15 strains, however both genes have to be present before denoted MRSA and the 4 cases of a negative test result were due to failure in detection of the SCCmec cassette junction.
Conclusion: A very rapid test (<3 hours) and easy to perform. The sequence targeted at the junction of the SCCmec cassette serves to ensure the *S. aureus* diagnosis and is a safeguard against false positive results. However, the current results raise the question whether genetic variation in the junction area reduces the sensitivity of the test. This study will be extended to a larger and more diverse collection of MRSA strains and patient swabs.

O26 Multicentre evaluation of laboratory work time and cost for real-time PCR tests for methicillin-resistant *Staphylococcus aureus* compared to microbiological culture

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Objectives: MRSA continues to increase globally. Rapid detection of MRSA colonization followed by appropriate isolation can reduce transmission and infection in healthcare settings (Ann Int Med 148:409–418, 2008). A concern raised is the cost of molecular assays plus the personnel time to perform this testing compared to culture. The BD GeneOhm™ MRSA (BD) test is a FDA cleared diagnostic assay for rapid detection of MRSA. Our objective was to compare personnel work time and cost needed for this assay and the LightCycler® MRSA Advanced test (Roche) to culture.
Methods: Double headed swabs were used to collect anterior nasal specimens from each subject. For the BD test, one swab was broken off in sample buffer tube, DNA was extracted and rt-PCR performed according to package insert. For the Roche test, DNA was extracted and rt-PCR performed according to proposed package insert. For culture, one swab was plated directly to CHROMagar™ as well as to an enrichment broth that was subsequently plated to CHROMagar™. Colonies resembling *Staphylococcus* species were confirmed as MRSA by standard methods. Processing time and cost of supplies were compared for these tests.
Results: Total hands-on technologist processing time for an average of 25 specimens was 75.4 min for the BD test (N = 263 runs) and 41 min for the Roche assay (N = 435 runs). The amplification and detection time

was 61.8 and 77.6 min, respectively. Hands on time for enriched culture processing of 25 specimens is estimated at 63 min (AJCP 131:532–9, 2009). Potential reporting time for a final result was <2 hours for the rt-PCR tests and 48.7 hours for enriched culture. The reagent cost for culture, including or excluding broth enrichment, is somewhat lower (8–10 Euro) than the rt-PCR tests from BD and Roche; costs of rt-PCR tests are comparable between these two suppliers. New rt-PCR methods have a comparable time of processing and cost of materials, but offer potentially much faster reporting time than does culture. The hands on time for the Roche assay was 1.8 min/test on average compared to 3 min with the BD assay and 2.5 min/test for culture.
Conclusion: The Roche and BD tests had little difference in overall time to result. With respect to resource utilization, hands-on time for the Roche assay was lower than BD and culture; the cost per test is comparable for both PCR assays. Molecular assays are able to report results more rapidly compared to culture with comparable resource utilization.

O27 A novel multiplex PCR identifies 7 staphylococcal species and mecA gene directly from blood cultures

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Objectives: Besides the well-established pathogenic potential of *Staphylococcus*. aureus, coagulase negative species virulence is increasingly being appreciated. The aim of the present report was to describe a multiplex PCR assay suitable for the identification of 7 staphylococcal species and the detection of the methicillin resistance determinant mecA.
Methods: Eight different primer pairs targeting femA (for *S. aureus*, *Staphylococcus hominis* and *Staphylococcus saprophyticus*), sodA (for *Staphylococcus haemolyticus* and *Staphylococcus capitis*), fbl (for *Staphylococcus lugdunensis*), a gene of unknown function (for *Staphylococcus epidermidis*) and mecA, were used for the optimization of the multiplex PCR protocol. The assay was tested on DNA extracted from solid cultures of 213 isolates belonging to 12 staphylococcal and 17 non-staphylococcal species (6 Gram-positive and 11 Gram-negative). RFLP analysis of the tuf gene was used as a reference method for speciation of 196 staphylococcal isolates, whereas the presence of mecA gene was assayed by a uniplex PCR. The 17 non-staphylococcal strains were speciated by Phoenix System (Becton Dickinson, BD). The assay was also tested on DNA extracted directly from 21 blood culture broths (Aerobic/F, Anaerobic/F, BD), spiked with isolates previously assigned to the staphylococcal 7 species (one reference strain and two clinical from each species) included in the multiplex protocol. An organic extraction protocol using benzyl alcohol was optimized for DNA extraction from the blood culture broths.
Results: The new method identified correctly 67 strains as *S. aureus*, 64 as *S. epidermidis,* 18 as *S. lugdunensis,* 16 as *S. haemolyticus,* 10 as *S. hominis,* 6 as *S. saprophyticus* and 4 as *S. capitis.* No amplification products were recorded for the remaining staphylococcal species and genera. In accordance with the uniplex mecA PCR, 61 methicillin resistant staphylococci were detected by the multiplex protocol. Application of the protocol on DNA extracted directly from spiked blood bottles produced the expected results in all cases within 6–8 hours.
Conclusion: The newly developed multiplex PCR assay specifically discriminates the 7 most commonly encountered staphylococcal species and concurrently determines their resistance towards methicillin. Its application on positive blood cultures is expected to reduce significantly the turn-around time and reliable identification and susceptibility results.

O28 Exotoxin-profiling of Panton-Valentine leukocidin positive MSSA versus MRSA

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Objective: The pathogenicity of *S. aureus* depends on the synergistic interaction of bacterial virulence factors. Accordingly, the emergence of virulent strains is frequently associated with the acquisition of genes encoding diverse virulence factors. As such, the recent spread of CA-MRSA was found associated with the presence of Panton Valentine (PVL) toxin. However, the exotoxin profile of PVL-positive MRSA versus MSSA is not known.
Methods: We analyzed a collection of 79 clinical isolates of PVL-positive MRSA and MSSA for the presence of genes encoding 22 exotoxins, including enterotoxins (ET) (sea-see, seg-ser, seu), toxic shock syndrome toxin (TSST)-1 (tst), exfoliative toxins (EF) (eta, etb) and hemolysines (HL) (hla) using PCR. Furthermore, MRSA genes (mecA, SCCmec) and agr-locus were investigated by PCR.
Results: In total we studied 61 MRSA and 18 MSSA strains. More than 90% of mecA positive MRSA harboured the SCCmec cassette SCCmec IV (71%) and 21% SCCmec V (21%), indicating the high incidence of CA-MRSA in the investigated collection. While agr3 was present in most MRSA and MSSA altogether (52%), agr4 was surprisingly only found in MSSA (56% of MSSA). Overall, MRSA harboured significantly fewer toxin genes as compared to MSSA. This holds true for enterotoxins, among which the genes for seg, sei, sem, sen, seo, and seu (= enterotoxin gene cluster egc) were the most prevalent ones (on average 30% of MRSA and 95% of MSSA tested positive for respective genes). Rather surprising, and in contrast to published studies from PVL-negative MRSA, we only discovered 12% of PVL-positive MRSA to express enterotoxin A (sea).
Conclusions: This exotoxin-profiling study revealed that the majority of PVL-positive *S. aureus* are CA-MRSA. Furthermore, the absolute number of expressed exotoxin genes was higher in PVL-positive MSSA versus MRSA, while 'common' MRSA-associated toxins such as enterotoxin A, were found in relative low frequency in PVL-positive MRSA.

O29 Different PVL encoding phages insert into the same chromosomal locus in distinct lineages of MRSA in England and Wales

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Objectives: To date, six Panton-Valentine Leukocidin (PVL) encoding phages (phiPVL, phiSLT, phiSa2mw, phi108PVL, phiSa2USA and phiSa2958) have been reported in PVL positive MRSA (PVL-MRSA). We sought to detect and analyse the DNA sequence of the chromosomal insertion site(s) of lysogenised PVL-phages amongst diverse PVL-MRSA clones found in England and Wales.
Methods: PCRs differentiated the lysogenised PVL-encoding phage present in PVL-MRSAs representing MLST Clonal Complexes (CCs) 1 (n = 11), 5 (n = 9), 59 (n = 3), 88 (n = 5) and ST93 (Queensland clone) (n = 11) as well as CC8 (USA300; n = 12) and CC80 (European clone; n = 7). Additional PCRs spanning the proximal and distal junctions of the phage / chromosome DNA were designed to detect the insertion site of the six known PVL phages and resultant DNA amplicons were sequenced on both strands.
Results: PCR and DNA sequence data indicated different PVL phages integrated at the same chromosomal locus in isolates of different lineages of PVL-MRSA. Lineage specific sequence polymorphisms in the chromosomal insertion site surrounding the PVL phage sequence occurred in isolates of CC 59 (SNPs = 8), 93 (SNPs = 5) and 5 (SNP = 1). By contrast, in CC1 and CC8 the insertion site sequence was identical to the USA300 genome. The PVL encoding phage was consistent within the lineage for: CC8 (phiSa2USA); CC80 (phiSa2MW); CC88 (Unknown Elongated Phage) and ST93 (phiSa2USA). Conversely, different PVL phages were identified within the same lineage for CCs; 1, 5 and 59. Polymorphisms in both ends of the phage genomes were detected and in most cases SNPs correlated with the identity of the PVL encoding phage. However, in some lineages the same SNPs occurred in apparently variant phages; in CC1 the same SNPs were identified in three different phages; phiSa2USA (n = 7) and phiSa2MW (n = 3) and unknown PVL phage (n = 1); whilst in CC5 a single SNP occurred at the 5′ end of either phiSa2USA (n = 7) or an unknown variant phage(s) (n = 2).
Conclusion: Amongst genetically diverse UK PVL-MRSA different PVL phages integrated at the same chromosomal locus. In some lineages

the DNA sequence at the phage insertion locus was specific to the PVL-MRSA lineage, we also detected SNPs in the extremities of the genomes of the lysogenised PVL phages. Together with MLST CC and SCCmec, these data suggest hitherto unidentified variance in lysogenised phages and suggests that lineages of PVL-MRSA have evolved on multiple occasions.

O30 Kinetics of bacterial DNA in blood during endovascular infections with *Staphylococcus aureus*

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Objectives: Endovascular infection with *Staphylococcus aureus* is a serious infection that requires rapid and adequate treatment to minimize the risk of metastatic foci. Currently there are no effective tools to monitor response to therapy. Sensitivity of blood cultures significantly decreases after initiation of antimicrobial therapy and other laboratory parameters such as C-reactive protein lack specificity. Measurement of bacterial DNA load (BDL) is not influenced by the use of antibiotics and has been shown to have a good correlation with severity of infection. To understand the kinetics of this parameter and to investigate its possible usefulness in guiding clinical management, we have prospectively determined the sequential BDL in patients with *S. aureus* endovascular infections.
Methods: Whole blood samples were collected from patients with culture proven *S. aureus* bacteraemia at several time points over a 2 week period. BDL was determined in 44 sequential samples from 8 patients with endovascular infection. We studied 4 severe endovascular infections (endocarditis and/or other intravascular foci) requiring more than 2 weeks of therapy, and 4 non-severe endovascular infections (phlebitis) requiring a maximum of 14 days of therapy. Quantification of *S. aureus* specific DNA was performed by real time PCR on DNA extracted from 200ul of blood.
Results: The *S. aureus* specific BDL was above the cut-off (5 cfu/ml) in 4 of 6 available samples taken within 24 hours after the first positive blood culture. Median BDL was 28 cfu/ml (range: 11–664). In all five patients who had detectable BDL levels during treatment, the load decreased significantly in the first days after treatment. In patients with non-severe infections, the BDL decreased below the detection limit within 4 days. In contrast, in patients with severe infections, the load remained detectable during the first 7 days. CRP measurements showed no correlation with disease severity.
Conclusion: We present the first description of kinetics of BDL in blood from patients with *S. aureus* endovascular infections. The results indicate that severe infections are characterized by prolonged presence of detectable BDL. These results provide new insights and suggest that BDL measurement has the potential to be used for monitoring treatment response.

O31 External quality assessment of molecular diagnostics of methicillin-resistant *Staphylococcus aureus*

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Background: This external quality assessment study determines the performance of molecular diagnostics for meticillin-resistant *Staphylococcus aureus* (MRSA) in the participating laboratories and was organised by Quality Control for Molecular Diagnostics (QCMD) (www.qcmd.org).
Methods: Eleven samples containing various concentrations of in-activated cells of MRSA, meticillin-susceptible *S. aureus* (MSSA), meticillin-resistant coagulase-negative staphylococci (MRCoNS) and *Escherichia coli* were distributed to 80 laboratories in August 2009.
Results: Out of the 80 participants, 68 (85%) responded. Samples containing 102 or 103 MRSA cells were correctly detected in only 16% and 46% of the datasets returned, respectively. Two MSSA panel strains contained a SCCmec cassette lacking the mecA gene. Only 12% of the datasets generated using commercial PCR kits, reported correct results for these two MSSA strains, which is a marked difference compared to the MSSA strain lacking the SCCmec cassette (89% correct results). The MRCoNS sample was correctly reported as negative in 89% of datasets generated using commercial tests used and by 70% of in-house assays. The *E. coli* sample was correctly reported as negative in 89% of datasets.
Discussion: In this EQA study on molecular diagnostics for the detection of MRSA, we conclude that the detection of MRSA by using samples with high CFU counts is reliable, which can and has been implemented in the laboratory setting with confidence. Pre-enrichment of clinical samples leads to concentrations of MRSA exceeding 109 CFU/ml, but also reduces one of the major improvements offered by NAAT, namely more rapid diagnosis.
This year, two MSSA samples harbouring a SCCmec cassette, but lacking the mecA gene, were included. The high percentages of incorrect results for commercial PCR underscore the need for improved specificity of these MRSA tests and therefore positive results should always be confirmed by a culture method or a second molecular test.
Performance in 2009 shows no significant changes since our first EQA in 2006. Major diagnostic performance discrepancies still exist between diagnostic microbiology laboratories.
In conclusion, the quality of molecular diagnostic MRSA tests still needs improvement. Programme expansion is required and regular quality control and international standardisation for MRSA diagnostics should be mandatory for the years to come.

Drugs and combinations for difficult-to-treat *S. aureus* infections

O32 A novel approach to identify antimicrobial synergy against methicillin-resistant *Staphylococcus aureus*

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The increasing occurrence of vancomycin (V) resistance among methicillin-resistant *Staphylococcus aureus* (MRSA) represents a serious therapeutic problem. Proposed empiric approaches to combination therapy have been based on little evidence.
Objectives: 1) To examine the pharmacodynamics (PD) of V paired with several antibiotics. 2) To identify synergistic combinations. 3) To develop better approaches to describe PD drug interactions.
Methods: Two MRSA strains Mu50 VISA and USA300 heteroresistant-VISA (USA300 hVISA) were used. MICs were completed as per CLSI. Time kill experiments were performed for V at 0, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 & 256 mg/L vs. each strain in log phase growth at two initial inocula: 10^6 & 10^8 CFU/mL. To accommodate the study of multiple antimicrobials a previously unexploited screening method was used employing the maximal effect (Emax) concentrations (guided by highest clinically achievable conc.) vs. two initial initial inocula for 6 pairs of antibiotics: V 128 mg/L + gentamicin (G), ciprofloxacin, TMP/SMX, caftazidime, tetracycline and linezolid. Combinations that demonstrated activity at screening were studied at 9 combinations for the same two drugs with concentrations ranging from low to Emax of each drug. PD analysis was performed by integrated area approach for reduction in area under the CFU vs. time curves, which were fit to 2-D or 3-D Hill-type PD models. We developed a new PD model which adheres to Loewe additivity and an interaction index (AFA index).
Results: Maximal bacterial reductions for V vs. 10^8 CFU/mL were −2.91 (−1.97) and −3.04 (−1.42) for Mu50 and USA300 hVISA at 48 h (24 h). For USA300 hVISA V+G was synergistic against 10^8 CFU/mL with max reductions of −7.85 (−7.85) at 48 h (24 h). 48 h log reductions for were: V32(−2.91) + G0.5(+1.4) = −3.28, V32 + G8(+1.58) = −6.45, V32 + G64(+1.64) = −7.84, V128(−3.03) +G0.5 = −6.26, V128 + G8 = −6.70, V128 + G64 = −7.85. AUC based PD parameters for V at 48 h/24 h/8 h were H = 4.60/3.57/3.68; Emax = 1.48/1.05/0.558; EC50 = 18/14.07/9.13; R^2 = 0.998/0.994/0.992. The same parameters for G at 8 h were H = 1.67, Emax = 1.51, EC50 = 3.55, R^2 = 0.996. Per the AFA index all V+G combinations studied for V > 1 mg/L demonstrate activity 400–500 fold greater than additivity (see fig. 1).

Conclusion: MRSA hVISA represents a therapeutic challenge with few alternatives. This study may have implications for optimal therapy of difficult to treat MRSA infections to preserve the usefulness of V if combined with other drugs.

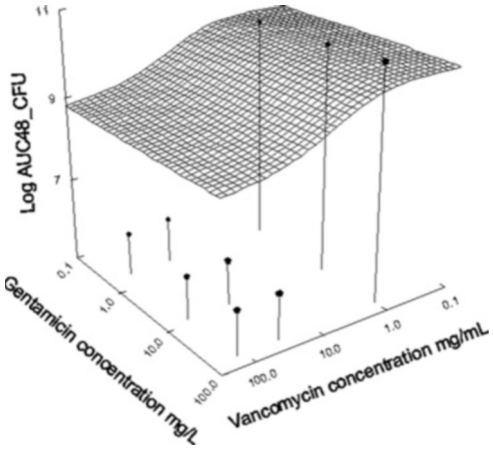

Figure 1. PD interaction surface analysis: vancomycin & gentamicin departures from additivity.

O33 Telavancin pharmacokinetics and pharmacodynamics in patients with complicated skin and skin structure infections with varying degrees of renal function

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Objectives: Telavancin is a lipoglycopeptide approved in the US and Canada for complicated skin and skin structure infections (cSSSI) due to Gram-positive bacteria, including MRSA. The objectives were to (1) simulate telavancin plasma concentration-time profiles in cSSSI patients with varying degrees of renal function and (2) evaluate the percentage of subjects with AUC/MIC ratios greater than the pharmacodynamic target of 219.
Methods: Data from 513 patients from telavancin phase 2–3 cSSSI clinical trials were used to perform the analysis. Individual concentration-time profiles were simulated for 10260 subjects (NONMEM VI, Icon, Elliot City, MD) using a previously described population two-compartment model, with a combined additive and proportional residual error model. The structural model was parameterized on clearance (CL), volume of the central compartment (V1), intercompartment clearance (Q), and volume of the peripheral compartment (V2). Distributions of body weight and creatinine clearance of the 513 patients were used to simulate body weight and creatinine clearance values for 10260 subjects in Matlab R2006a (Mathworks, Natick, MA). Telavancin dosing regimens simulated were 10 mg/kg Q24H for creatinine clearance (CrCl) > 50 mL/min, 7.5 mg/kg Q24H for CrCl 30–50 mL/min and 10 mg/kg Q48H for CrCl < 30 mL/min. AUC under one dosing interval (AUCtau) was computed as dose/CL. The number of subjects achieving an AUCtau/MIC ratio of 219 or greater was evaluated separately in subjects with mild renal impairment to normal renal function (CrCl > 50 mL/min), moderate renal impairment (CrCl between 30–50 mL/min), and severe renal impairment (CrCl < 30 mL/min), using MIC values of 0.5, 1, and 2 mg/L.
Results: Summary statistics of AUCtau values of subjects with varying degrees of renal function are provided in the table. Using the 3 dosing regimens, AUCtau values were similar across the renal function groups. More than 90% of the simulated subjects achieved an AUCtau/MIC ratio of 219 or greater, assuming an MIC of 0.5, 1 or 2 mg/L (targeting 2× and 4× the MRSA MIC90 of 0.5 mg/L).
Conclusion: Using the three dosing regimens, AUCtau were similar across the different renal function groups, indicating that the dose adjustments employed in the phase 3 cSSSI trials were appropriate. All proposed telavancin dosing regimens are expected to provide an AUCtau/MIC ratio of 219 or greater in at least 90% of the population, for organisms with an MIC of 2 mg/L or less.

Simulated AUC_τ and the AUC_τ/MIC ratio in subjects with various degrees of renal function

Parameter	CrCl (mL/min)		
	<30†	30–50#	>50‡
AUC_τ (mean±SD, mg.h/L)	1058±316	762±238	776±264
AUC_τ range (min, max, mg.h/L)	(466, 2071)	(318, 1974)	(203, 2820)
Percentage of population with AUC_τ/MIC ⩾219			
MIC = 0.5	100%	100%	100%
MIC = 1	100%	100%	100%
MIC = 2	98.6%	95.0%	93.8%

†Based on 140 simulated profiles; #based on 480 simulated profiles; ‡based on 9640 simulated profiles.

O34 Biphasic killing of levofloxacin against *Staphylococcus aureus*: modelling bacterial response to drug-selective pressures

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Objective: We have previously developed a mathematical model predicting the response of *P. aeruginosa* to levofloxacin (LEV) (Ann Biomed Eng 07). However, the applicability of the model to other pathogens is unknown. We extended our model to predict the effect of various fluctuating LEV exposures on *Staphylococcus aureus* (SA), a bacterium commonly associated with nosocomial bloodstream and surgical site infections.
Methods: Time-kill studies (TKS) with 10^7 CFU/ml of SA ATCC 29213 at baseline were performed in duplicate. LEV at 0–16 mg/l was used for 24 h (MIC = 0.25 mg/l). The experimental data were used to derive estimates of the best-fit model parameters, and SA response (resistance development or suppression) to various LEV exposures was predicted via a 3-dimensional response surface. The computer model predictions were subsequently validated using an *in vitro* hollow fiber infection model (HFIM); LEV profiles (t1/2 = 7 h) corresponding to different daily doses (given every 12 h or 24 h) were investigated over 120 h.
Results: In contrast to the anticipated concentration-dependent killing, LEV exhibited a biphasic killing profile in TKS. The data could only be satisfactorily captured by a modified mathematical model ($r^2 = 0.977$). Dosing frequency was predicted not to have significant impact on bacterial response, as long as the daily doses were kept constant. A daily dose of <300 mg was predicted to facilitate resistance development, while >500 mg was expected to suppress resistance. These predictions correlated well with our experimental data in HFIM.
Conclusions: The modified mathematical model was reasonable in predicting extended SA response to various fluctuating LEV exposures qualitatively, based on limited input data from TKS. In view of its robustness and efficiency, our mathematical modeling approach could be used as a decision-support tool for dosing regimen selection in antimicrobial (pre)-clinical development.

O35 Penetration of daptomycin in serum and bradytroph tissues of patients undergoing orthopaedic surgery

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Objective: To investigate the serum and tissue concentrations of daptomycin in patients undergoing orthopaedic surgery.
Methods: A total of 18 patients received 350 mg of daptomycin in a short intravenous bolus infusion. Three groups of six patients each received the drug prior to hip- or knee replacement surgery, i.e. between 1–1.5 h (Group I), between 1.5–2.5 h (Group II) and between 2.5–3.5 h (Group III) before intra-operative sampling of serum and tissue, i.e. bone, cartilage, muscle, fat, was performed.

Results: Daptomycin serum concentrations showed a mean of 62.5 [μg/ml] in group I, 45.2 [μg/ml] in group II and 31.9 [μg/ml] in group III. In bone a mean of 5.3 [μg/g] in group I, 4.5 [μg/ml] in group II and 4.5 [μg/ml] in group III was found. Cartilage showed a mean of 1.9 [μg/g] in group I, 2.0[μg/ml] in group II and 1.7 [μg/ml] in group III. Muscle revealed a mean of 4.0 [μg/g] in group I, 2.3 [μg/ml] in group II (for 5 patients) and 2.6 [μg/ml] in group III. In fat only a few samples could be successfully analysed. These showed a mean of 6.0 [μg/g] in group I (evaluable samples only for two patients), a mean of 1.9 [μg/ml] in group II (evaluable samples only for three patients); for group III the samples of all six patients were below the detection limit of the analytical method. In a linear regression analysis the decrease of serum daptomycin concentration per minute was estimated as –0.230, 95% confidence interval (CI) [–0.347, –0.112], p = 0.0008. For bone concentrations the decrease was –0.0078, 95%-CI [–0.0247, 0.0092], p = 0.35) including all 18 patients, and –0.012, 95% CI [–0.024, –0.0005], p = 0.042) excluding one single patient with an extraordinary high value. For cartilage, muscle and fat, no decrease over time was shown. However, for serum, bone, cartilage, muscle and fat the daptomycin concentrations were clearly above the MIC values for staphlococci (<1.0 μg/ml).
Conclusions: Daptomycin concentrations in serum showed a slow decrease and the drug concentrations measured in both serum and bradytoph tissues remained clearly above the MICs for staphylococci for up to 3.5 hours. Within the postoperative observational period of one week, no postoperative wound infection occurred. The application of daptomycin was well tolerated without any adverse effects.

O36 Vancomycin serum trough levels and outcomes in patients with hospital-acquired pneumonia due to *Staphylococcus aureus*: the ATTAIN study

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Objectives: Higher vancomycin (VAN) serum trough levels (STL) have been recommended when MRSA HAP is suspected. However, the correlation between VAN STL and clinical outcomes and safety is still poorly understood.
Methods: ATTAIN 1 and 2 were randomized, methodologically identical, double-blind, phase 3, clinical trials in patients with hospital-acquired pneumonia (HAP). We analyzed baseline characteristics and outcomes from patients with *S. aureus* (SA) randomized to VAN (1 g IV q12 h; 7 to 21 days) as a function of median VAN STL. VAN could be adjusted for renal function and/or institutional policies. The all-treated (AT) population included randomized patients who received ⩾1 dose of study medication. This analysis was limited to the subset of SA-infected patients with VAN STL.
Results: 98 VAN AT patients with S aureus had STL (Table).

	Median VAN Trough Level (μg/ml)			
	<10 (n = 30)	⩾10 to <15 (n = 33)	⩾15 (n = 35)	p-value [1]
Number of trough values per patient (median, range)	1 (1 to 5)	2 (1 to 8)	2 (1 to 14)	–
Age (mean±SD) (yr)	60±19.6	69±12.7	69±13.6	0.139 (KW)
ICU at baseline	20 (67%)	24 (73%)	28 (80%)	0.485 (chi)
APACHE (mean±SD)	16±5.6	17±6.6	18±6.0	0.452 (KW)
VAP	13 (43%)	13 (39%)	14 (40%)	0.934 (chi)
Organism				
MRSA	20 (67%)	26 (79%)	30 (86%)	0.186 (chi)
MSSA	10 (33%)	7 (21%)	5 (14%)	
MRSA MIC [3]				
0.5 μg/mL	1/18 (6%)	3/22 (14%)	5/23 (22%)	0.354 (chi)
1.0 μg/mL	17/18 (94%)	19/22 (86%)	17/23 (74%)	[2]
>1.0 μg/mL	0/18 (0%)	0/22 (0%)	1/23 (4%)	
MSSA MIC [4]				
0.5 μg/mL	2/9 (22%)	1/6 (17%)	1/4 (25%)	1.000 (chi)
1.0 μg/mL	7/9 (78%)	5/6 (83%)	3/4 (75%)	
Clinical response of "cure"	21 (70%)	18 (55%)	17 (49%)	0.104 (CA)
Any serious TEAE	4 (13%)	8 (24%)	13 (37%)	0.033 (CA)
Renal TEAE	0 (0%)	1 (3%)	6 (17%)	0.006 (CA)
Deaths	3 (10%)	5 (15%)	7 (20%)	0.306 (CA)

ICU, intensive care unit; SD, standard deviation; TEAE, treatment-emergent adverse event; [1] 2-sided p-value from the Kruskal-Wallis test ("KW"), an exact Pearson chi-square test ("chi"), or an exact Cochran-Armitage trend test ("CA"); [2] Test of 0.5 mcg/mL vs. ⩾1.0 μg/mL.

Conclusions: Patients in whom higher VAN STL were achieved had poorer outcomes; and were more likely to experience renal adverse events than patients with lower VAN STL.

O37 Combination therapy with cefoxitin and β-lactams is synergistic against community-associated but not hospital-acquired methicillin-resistant *Staphylococcus aureus* strains *in vitro*

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Objective: There is an urgent need for strategies to treat community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections. Preliminary *in vitro* studies demonstrate that nafcillin minimal inhibitory concentrations (MICs) for USA300 and USA400 strains decrease in the presence of subinhibitory concentrations of cefoxitin. The aim of this study was to characterize the *in vitro* antimicrobial activity of cefoxitin and several anti-staphylococcal β-lactams alone and together against MRSA isolates using MICs and time kill assays.
Methods: MICs and time kill assays were performed using standard methods with the following strains: ATCC29213 (control strain), USA300 and USA400 (CA-MRSA strains), N315 and COLn [hospital-acquired (HA) MRSA strains]. Time kill assays were performed with a starting inoculum of 5.5 to 6.5 log10 CFU/ml and quantitative cultures performed at 0, 6 and 24 h.
Results: The MICs of nafcillin, cefazolin, cefuroxime, ceftriaxone and cefotaxime decreased in the presence of 10 ug/ml cefoxitin to a greater extent for CA- than HA-MRSA strains (see table). In triplicate time kill assays, cefoxitin (10 ug/ml) combined with either nafcillin (4 μg/ml) or cefazolin (16 μg/ml) displayed synergy against CA- but not HA-MRSA strains. Compared to nafcillin alone, nafcillin plus cefoxitin showed a log10 CFU/ml reduction ranging from 0.8 to 2.1 for USA300 and 1.5 to 2.1 for USA400. Compared to cefazolin alone, cefazolin plus cefoxitin showed a log10 CFU/ml reduction ranging from 2.8 to 2.9 for USA300 and 2.9 to 4.7 for USA400. Overall, cefazolin plus cefoxitin caused a log10 CFU/ml reduction of the initial inoculum at 24 h ranging from 0.8 to 1.2 for USA 300 and 1.6 to 3.1 for USA400. The activity of cefazolin combined with cefoxitin was superior to that of clindamycin (8 μg/ml) alone but inferior to that of vancomycin (16 μg/ml) alone for USA300 and USA400.
Conclusion: Cefoxitin combined with a variety of β-lactams enhanced their activity against CA-MRSA strains *in vitro*. Cefoxitin synergy was greater with cefazolin than with nafcillin. Combination β-lactam therapy for CA-MRSA deserves further study.

	Minimal inhibitory concentration (microg/ml)*						
Strain	Cefoxitin	Nafcillin	Cefazolin	Cefuroxime	Ceftriaxone	Cefotaxime	Vancomycin
USA300	32	4/0.5	16/0.5	64/2	128/16	32/8	1/1
USA400	32	4/0.5	4/0.5	>128/2	128/8	32/2	1/1
N315	32	16/8	64/1	>128/>128	>128/32	128/8	0.5/1
COLn	>128	128/>128	>/128/>128	>128/>128	>128/>128	>128/>128	0.5/1

*Antibiotic/Antibiotic plus 10 microg/ml cefoxitin.

O38 Evaluation of vancomycin and oxacillin combination against mecA positive vancomycin-intermediate *Staphylococcus aureus* (VISA) and heterogenous VISA with varying oxacillin susceptibility

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Objectives: *S. aureus* with reduced susceptibility to vancomycin (VAN) currently represents a serious clinical dilemma given the limited availability of alternative treatments. The VAN intermediate-type of resistance in *S. aureus* (VISA) has been previously reported to result in cell wall remodeling and antimicrobial susceptibility changes in favor of b-lactam activity. We evaluated the activity of VAN, oxacillin (OXA) and cefoxitin (CFX) and the potential for synergy of the combination of VAN+OXA against a collection of clinical heterogenous VISA (hVISA) and VISA strains.

Methods: 60 VISA and 93 hVISA isolates carrying the mecA genes were selected from the Anti-Infective Research Laboratory collection. VAN, OXA and CFX MICs were determined in duplicate by broth microdilution according to CLSI guidelines. Pearson's rank correlation coefficient test was used to assess the association between VAN and OXA or CFX MICs. Eight VISA and hVISA strains were selected on the basis of their OXA MIC to be evaluated by time kill curves (TK) against VAN and OXA alone or in combination at 0.5 and 0.25× MIC in presence of 50% human serum. Synergy (S) was defined as ⩾2 log kill compared to the most efficient drug alone.
Results: MIC distribution is reported in Table1. Of interest, 23% and 10% of the VISA strains were susceptible to OXA and CFX, respectively. A significant inverse correlation was found between the MICs of VAN and OXA (P = 0.003), and VAN and CFX (P = 0.001). Considering the subpopulation with the highest VAN MIC for each hVISA strain, 36% displayed a 2–8 x decrease in the OXA MIC compared to the overall population. In contrast, 41% had a 2–16× increase in OXA MIC. Isolates performed in TK exhibited a MIC range of 2–8 mg/L and 0.5–512 mg/L for VAN and OXA, respectively. In TK, S was observed with the combination of VAN+OXA at 0.5 x MIC against all VISA and hVISA isolates, except 1 VISA strain resistant to OXA and 1 hVISA exhibiting a decreased OXA MIC in the population with the highest VAN MIC. Combination of VAN+OXA at 0.25 x MIC did not demonstrate S against any tested isolates.
Conclusions: We confirmed susceptibility changes in favor of b-lactam activity in the VISA collection. Further *in vitro* and *in vivo* experiments are now warranted to determine if the potentially synergistic combination of VAN+OXA would be useful to treat patients with infections caused by MRSA, in order to eradicate the infection and/or prevent emergence of VAN intermediate resistance.

Table 1. MICs distribution of 93 hVISA and 60 VISA isolates.

Species	Percent of isolates									
	VAN MIC (mg/L)				OXA MIC (mg/L)			CFX MIC (mg/L)		
	0.5	1	2	4–8	⩽4	8–32	64–>512	⩽16	32–64	128–>512
*h*VISA (*n* = 93)	4	66	30	0	0	13	87	1	26	73
VISA (*n* = 60)	0	0	0	100	23	25	52	10	48	42

O39 *In vitro* synergism between ceftobiprole and vancomycin against methicillin-resistant and glycopeptide-intermediate *Staphylococcus aureus*

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Objective: Several studies have reported synergy *in vitro* between β-lactams and vancomycin (VAN) in their activity against methicillin-resistant *S. aureus* (MRSA) and glycopeptide-intermediate *S. aureus* (GISA) isolates. Nevertheless, attempts to use such combinations against GISA in animal infection models yielded conflicting results. This was probably due to the fact that the β-lactams used (e.g., cloxacillin and nafcillin) had weak affinity for penicillin-binding protein 2A (PBP2A), the major determinant of methicillin resistance in MRSA and GISA. Ceftobiprole (BPR) is a novel cephalosporin with improved affinity for PBP2A, and enhanced *in vitro* activity against MRSA and GISA. Here we tested the potential for synergy between BPR and VAN against both MRSA and GISA *in vitro*.
Methods: Three GISA clinical isolates (Mu50, PC3 and 1092, a human isolate from Switzerland) were tested. One VAN-susceptible MRSA (COL) and one VAN-susceptible methicillin-susceptible *S. aureus* (MSSA 1112) were used as controls. The interaction of BPR with VAN was determined by applying BPR or VAN Etest strips to inoculated (0.5 McFarland) BHI agar plates with the partner drug incorporated into the agar at 0, 0.25× and 0.5× the MIC for each strain. The time-kill assay was used to test for BPR and VAN synergy using antibiotic concentrations of 0.25× to 1× the MIC.
Results: MICs of BPR were 1–2 mg/L for GISA, 1 mg/L for MRSA, and 0.12 mg/L for MSSA. MICs of VAN were 4–8 mg/L for GISA, and 2 mg/L for both MSSA and MRSA. Subinhibitory concentrations of VAN added to the agar medium caused 2- to 4-fold decreases in BPR MICs for GISA and 4-fold for MRSA. The GISA and MRSA isolates showed corresponding 4- to 8-fold decrease in VAN MIC in the presence of subinhibitory levels of BPR. In time-kill curves, combinations of BPR and VAN displayed synergism (defined by a decrease of ⩾2log10 CFU/ml at 24 h compared with the single most active agent and with the starting inoculum) for all GISA and MRSA strains. For MSSA, the combination was indifferent by both Etest and time-kill methods.
Conclusions: *In vitro*, BPR and VAN were synergistic against GISA and MRSA. An indifferent effect was noted against MSSA. Assessment of BPR plus VAN for the therapy of GISA in an experimental animal model of infection (experimental endocarditis) is currently in progress to assess the potential clinical therapeutic benefit of this combination.

O40 Comparative *in vitro* activity of torezolid and linezolid against *Staphylococcus* and *Enterococcus* isolates

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Objective: Torezolid is a new oxazolidinone with *in vitro* and *in vivo* efficacy against several Gram-positive species. The aim of our study was (i) to determine Linezolid (LZ) MICs against 104 French isolates of *Staphylococcus* and *Enterococcus* (ii) to characterize LZ resistance mechanisms for the strains with a LZ MIC >2 mg/L (iii) to compare the *in vitro* activity of Torezolid with LZ.
Methods: A total of 104 French isolates of animal and clinical origin resistant to chloramphenicol were studied including 38 *Staphylococcus* strains and 66 *Enterococcus* strains. MICs of LZ and Torezolid were determined using the Mueller Hinton agar dilution method according to CLSI guidelines. 23s rRNA target mutations and cfr genes were detected by PCR and sequencing.
Results: For all 104 strains, MICs of LZ were 0.5–64 mg/L (geom. mean MIC 7.3 mg/L). MIC of LZ was >2 mg/L for 23 strains: 6 *S. aureus* isolates and 17 *Enterococcus* isolates (12 *E. faecalis* and 5 *E. faecium)*, all of clinical origin. All *S. aureus* isolates resistant to linezolid harbored a G2576T mutation in rrl gene. *Enterococcus* isolates harbored either G2447T (n = 4), G2505A (n = 3) or G2576T (n = 10), No strains whatever their susceptibility to LZ harbored cfr gene.
For all 104 strains, MICs of Torezolid were <0.5–8 mg/L (mean MIC 1 mg/L. MICs of Torezolid were <0.5–1 mg/L vs. 0.5–2 mg/L LZ for LZ-susceptible staphylococci (n = 32) irrespective of species and methicillin resistance. Against linezolid susceptible *Enterococcus* of animal origin (n = 34) and clinical origin (n = 15), MICs of Torezolid were 0.5–1 mg/L vs. 2 mg/L LZ. MICs of Torezolid for LZ-resistant *Enterococcus* isolates were 1–8 mg/l vs. 4–64 mg/L LZ.
Conclusion: No cfr genes were found in French chloramphenicol-resistant *Staphylococcus* and *Enterococcus* isolates of human or animal origin. Torezolid was 4 to 8 fold more active than Linezolid against LZ-susceptible strains and LZ-resistant *Staphylococcus* and *Enterococcus* isolates with G2576T, G2505A or G2447T mutations.

O41 *In vitro* evaluation of the bactericidal activities of MRSA active antibiotics in four different peritoneal dialyses fluids

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Objectives: Continuous ambulatory peritoneal dialysis used in the treatment of patients with end-stage renal failure is often complicated by peritonitis. *Staphylococcus aureus* peritonitis is associated with severe peritonitis, particularly if caused by methicilin resistant strain (MRSA). The intraperitoneal administration (IP) of drugs for peritonitis is preferable to intravenous or oral administration because of the resulting higher local antibiotic concentrations. Peritoneal dialyses fluids (PDFs) affect bacteriostatic, which may compromise the effectivity of antibiotics. Therefore, it is important that prescribed antibiotics are compatible

with PDFs. The purpose of this study was to investigate *in vitro* the bactericidal effectiveness of for MRSA infections appropriated antibiotic in diverse PDFs.
Methods: Against MRSA the bactericidal activities of vancomycin (VAN), teicoplanin (TEI), daptomycin (DAP), linezolid (LIN), cefto-biprole (CEF) and tigecycline (TIG) in different PDFs: Dianeal PD4 Glucose 1.36%; Physioneal 40 Glucose 1.36%; Extraneal 7.5% Icodextrin, and Nutrineal PD4 1.1% amino acid were proved. Cation-adjusted Mueller-Hinton Broth. (CAMHB) was used as a control broth. Ten milliliter of diverse PDFs and CAMHB containing bacteria inoculum of approximately 106 CFU/ml was incubated for 2 h at 37ºC. Following incubation, the antibiotics at concentrations: 1 × MIC, 4 × MIC, 8 × MIC were added. Additionally, the bactericidal concentration at clinical used concentration: VAN 50 μg/ml, TEI 20 μg/ml, DAP 60 μg/ml, CEF 30 μg/ml, LIN 32 μg/ml, TIG 50 mg/ml was tested. Samples were taken at 2, 4, 6, 8 and 24 h and the number of CFU/ml was determined. To stimulate *in vivo* conditions human serum albumin at 2 g/l was added. Control experiments with bacteria and no antibiotics in PDFs and CAMHB were run.
Results: All antibiotics showed concentration- and time-dependent bactericidal activities, but the reduction of CFU/ml in diverse PDFs differed considerably. All tested antibiotics showed significantly higher bactericidal activity in Extraneal 7.5% Icodextrin, and Nutrineal PD4 1.1% amino acid than in Dianeal PD4 Glucose 1.36% and Physioneal 40 Glucose 1.36% ($p < 0.05$). The highest decrease in CFU/ml attained in Extraneal 7.5% Icodextrin, and Nutrineal PD4 1.1% amino acid showed DAP and VAN.
Conclusion: Based on these *in vitro* data, we conclude that the choice of PDFs used for IP administration is not trivial and could be crucial for therapy outcome.

Imported infections: tropical and travel medicine

O42 Investigation of molecular diagnostic assays for the detection of *Trypanosoma cruzi* DNA in blood

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Objectives: Chagas disease, caused by *Trypanosoma cruzi*, is endemic to Latin America, and of emerging importance in non-endemic countries because of migration of people infected with *T. cruzi.* The majority of patients diagnosed in non-endemic settings are in the indeterminate or chronic phases. However, acute cases may be seen in congenitally-infected infants and in people receiving blood products or organs from infected donors. Molecular diagnostic assays needed to be standardized to diagnose and monitor congenital infection, aid serological diagnosis in expatriate travellers and migrants from Latin America, and monitor infection in known cases of Chagas Disease.
Methods: Two molecular assays for the diagnosis of *T. cruzi* infection were investigated, a SYBR green real-time PCR targeting nuclear satellite DNA, and a hotstart PCR targeting kinetoplast DNA (kDNA). One hundred and eighty PCR controls and 180 blood-simulated specimens prepared from cultures of *T. cruzi* strains of lineages TcI, TcIIb and TcIIe were evaluated. Samples for DNA were prepared from the specimens using 3 lysis methods (Qiagen (Q), Guanidine-EDTA (GE) and GE+boiling (GEB). DNA extraction after lysis was carried out using silica-membrane technology. Ten-fold serial dilutions were prepared. Statistical analysis was performed by SPSS v15, Chicago IL.
Results: The sensitivity of the real-time PCR assay was calculated by PROBIT analysis with 12 replicates of 8-fold serial dilutions of the DNA from culture of TcIIb extracted by Qiagen. The 95% and 50% positive hit rates were 0.8 parasites/ml (95% IC: 0.41–1.53) and 0.2 parasites/ ml (95% IC: 0.08–0.32), respectively. The assay showed a specificity of 100%. More consistent results were found for all lineages and types of samples when DNA was extracted by the Qiagen method. A higher detection limit was found in lineage TcI ($p < 0.01$). Lineage TcIIb showed statistically better results in controls and simulated specimens, than the other two lineages ($p < 0.01$). The kDNA assay was performed in simulated specimens from the three lineages, and it gave better results using Q and GEB methods on low concentration samples, although not statistically significant ($p = 0.08$).
Conclusions: Molecular assays are very promising in the diagnosis of *T. cruzi* infection and have applications where serological assays are not of use. Further work should be done to standardize the methods.

O43 Congenital transmission of Chagas' disease in Latin American immigrants in a health department of Valencia, Spain

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Objective: To describe congenital transmission of Chagas disease in Latin American immigrants in our Health Department 07-La Fe, in Valencia (Spain).
Methods: We collected sera from pregnant women from Latin America who attended our hospital between June 2007 and October 2009. The samples were tested for anti-*Trypanosoma cruzi* antibodies (IgG) using 2 differents enzyme-linked immunosorbent assays (ELISA) and a particle gel immuno assay. Positive sera were then confirmed with an immunofluorescent assay (IFA). Total blood from infected patients was sent to Carlos III Health Institute (Madrid) in order to perform a polymerase chain reaction (PCR) study. Newborns' sera and umbilical cord blood from infected mothers were tested for anti-*T. cruzi* antibodies and blood microscopic examination, microhematocrit concentration technique and PCR, respectively, at birth, at 1–6-12 months old. Infected children, considering 2 positive PCR and/or positive parasitological examination for diagnosis, were treated with a 60-day course of benznidazol (3.5–7 mg/kg/day).
Results: Out of 574 pregnant women sera, 35 (6.1%) were positive for *T. cruzi* serology and gave birth at our hospital. Their mean age was 29.1 years old; All of them were from Bolivia, mainly from Santa Cruz (39.3%) and Cochabamba (28.6%) departments, except one from Argentina. 12 pregnant women PCR were positive, but none of them was symptomatic for Chagas disease. 2 newborns had two positive PCR along the first 6 months and 1 newborn had positive blood examination also. Two of them were born from positive PCR women and the other is unknown. Only one of the babies had symptoms at birth (dilated cardiomyopathy and a neuroblastoma). Benznidazol treatment was well tolerated by all babies.
Conclusions:
1. Although we have not documented any congenital transmission in newborns from negative PCR mothers, we cannot consider yet that positive PCR is a predictor factor for *T. cruzi* infection transmission.
2. Positive PCR results at birth allows us to initiate an early treatment in infected newborns, which has been demonstrated as the most effective treatment.
3. Benznidazol has been well tolerated by newborns with no side effects.
4. It is really important to follow-up seropositive children at least during one year with serology tests. Negative PCR blood from newborns only can be confirmed as non-infected patients when *T. cruzi* antibodies desapear along the first year.

O44 Paediatric drug formulations of artemisinin combination therapies: is there evidence for improvement of patient management in the treatment of children suffering from malaria? A systematic review and meta-analysis

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Objectives: Artemisinin combination therapy (ACT) is the mainstay of antimalarial treatment with high efficacy, good tolerability, and a reduced risk for resistance selection. However, conventional fixed dose ACTs are inadequate for the treatment of young children – the most important target population – due to difficulties in drug administration of tablets. Recently, a number of ACTs with paediatric

drug formulations have been developed. However to date there is no objective evidence from individual controlled trials for an improvement of patient management by their use when compared to conventional tablet formulations. We therefore aimed to collate all available evidence from individual controlled trials in order to investigate whether objective evidence supports the development of these paediatric antimalarials.
Methods: We performed a systematic review and meta-analysis of controlled clinical trials evaluating paediatric artemisinin combination therapies compared to tablet drug formulations. Outcome parameters were efficacy, safety, tolerability, and tolerability of drug administration.
Results: Out of 667 potentially relevant publication, seven studies met the predefined inclusion criteria. Meta-analysis of 2515 children was performed evaluating paediatric drug formulations of the following ACTs: artesunate-mefloquine, artemether-lumefantrine, and pyronaridine-artesunate. Per protocol and intention to treat analysis of efficacy were comparable between paediatric and tablet drug formulations. Overall tolerability, defined as the total number of adverse events experienced by patients was also similar between groups. However, the tolerability of drug administration evaluated by the number of drug related adverse events (RR: 0.79, 95% CI: 0.64–0.96), drug related vomiting (RR: 0.77, 95% CI: 0.61–0.99), and drug related gastrointestinal disorders (RR: 0.34; 95% CI: 0.15–0.80) was considerably improved for paediatric drug formulations.
Conclusion: Data of this meta-analysis show for the first time objective evidence of an improved tolerability of drug administration by paediatric ACTs compared to tablet ACTs. To our knowledge this is the first evidence for an improvement in patient management by paediatric drug formulations in any indication. Therefore paediatric ACTs should be considered in international recommendations for the treatment of malaria in young children.

O45 Is benign tertian malaria actually benign?

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Objective: This retrospective study was conducted to determine the incidence of various complications of *Plasmodium vivax* malaria based on review of case records in a tertiary care hospital, New Delhi, India.
Methods: The case records of all confirmed cases of malaria over the period of one year (September 2008-August 2009) were studied. Complete blood count, peripheral blood findings, liver and kidney functions were reviewed. The results of rapid diagnostic test for malaria (OptiMAL test, Diamed AG, Switzerland) were correlated with the peripheral blood smear findings in the patients in whom it was requested. All abnormal results like a positive direct Coomb's test were noted. Findings were clinically correlated.
Results: There were 165 confirmed cases by peripheral blood examination. Of these 121 were due to *Plasmodium vivax* and 42 due to *P. falciparum.* Two cases had mixed infection. The peak incidence of malaria was seen in September 2008 and July 2009. The complications in *P. vivax* were thrombocytopenia, biochemical evidence of hepatic dysfunction, renal damage, positive DCT and death due to ARDS. Thrombocytopenia was seen in 113 patients with counts $<20\times10^3$/microl in 43 patients. Fifteen patients had serum bilirubin >3 mg/dl with normal liver enzymes. Liver enzymes were elevated in 55 patients with twelve patients showing liver enzymes level, three times the normal. Renal dysfunction was seen in 21 patients with serum creatinine ranging from 1.3–10.65 mg/dl. There were three deaths due to ARDS.
Conclusion: This paper is presented to highlight that *P. vivax* malaria though considered to be a benign entity can also have a severe and complicated course which is usually associated with *P. falciparum* malaria.

O46 Incidence of influenza, dengue and Japanese encephalitis in Australian travellers visiting South and south-eastern Asia

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Objective: To estimate the incidence density of influenza, dengue and Japanese encephalitis (JE) in Australian travellers to Asia.
Methods: Prospective cohort study of Australian travellers to South and South East Asia over a 2 year period. Travellers ⩾16 years of age were recruited from 3 travel clinics, completed validated questionnaires and provided pre and post-travel blood samples for serological testing. Demographic data, destinations and travel patterns, vaccination details and history of flavivirus infection were obtained. Serological testing for dengue IgG by ELISA (Pan-Bio assay), Influenza A and B (complement fixation antibody) and JE (In-house indirect fluorescence antibody) was performed.
Results: Among 450 travellers enrolled, 345 have returned for follow-up, 53 (11.7%) have been lost to follow-up and paired sera have been tested for 324 travellers; 58% were female, median age was 32 years and 24% were born overseas. 72% were short term travellers (<30 days) and main traveller types were vacation/holiday goers (69%) and business travellers (16%). 76% reported prior travel to Asia and 10.8% and 54% had received the JE and influenza vaccines respectively. Dengue sero-prevalence: Acute seroconversion for dengue virus infection was demonstrated in 4/324 (1.2%) of travellers tested. This translates to an incidence of 4.17 dengue virus infections per 10,000 days of travel (95% CI 1.7–10.7). A further 13 travellers (4%) were positive for dengue IgG prior to travel indicating past exposure. Travellers with acute dengue infection had travelled to China (n = 2) India (n = 1) and Thailand (n = 1), and two of these travellers had received the JE vaccine. Influenza sero-prevalence: 4/324 (1.2%) had evidence of recent influenza infection. The incidence of influenza virus infections is 4.17 per 10,000 days of travel (95% CI 1.7–10.7). JE sero-prevalence: There was no acute JE seroconversion in this cohort.
Conclusion: To our knowledge, this is the largest prospective study estimating the incidence of both respiratory and arboviral infections in travellers. For travellers to Asia, the risk of acquiring dengue in an inter-epidemic period is low (incidence density 4.17 infections per 10,000 days of travel). The risk of acquiring influenza in this well vaccinated cohort was equally as low and no JE infections were observed. These findings have important implications for practitioners advising prospective travellers.

O47 Genetic diversity of intestinal protists: the implication for PCR-based diagnostic assays

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Objective: Diagnosis of intestinal protists by PCR is being employed more and more by diagnostic and research laboratories. Data from molecular characterisation of intestinal protists is the raw material for our current and future efforts to develop improved diagnostic PCRs. Apart from our ability to sample correctly and extract DNA from parasites directly from faeces, important issues include primer specificity and sensitivity, which have impact on predictive values of the diagnostic assays. Thus, it is important to be aware of the genetic diversity among these parasites. Extensive data from the molecular characterisation of intestinal protists are necessary if PCR-based diagnosis is to detect all genetic variants of intestinal parasites especially if the eventual aim is for it to substitute for morphology-based methods. Diagnostic PCRs are very often based on amplification of the Small Subunit (SSU) rRNA gene, partly due to the fact that this gene is present in a high-copy number. However, data from very few strains are currently available for the SSU rRNA gene of most intestinal parasites. Often only one or two sequences are available in the Genbank database.
Methods and Results: We are in the process of collecting data from the genetic characterisation of protozoa such as Iodamoeba, *Entamoeba coli*,

Entamoeba hartmanni and Entamoeba polecki-like organisms isolated from clinical samples. Knowing the amount of diversity displayed among clinical isolates of these (and other) parasites will help us generate specific and sensitive primers that can be used in cases where a definitive diagnosis cannot be established on the basis of microscopy. Preliminary results show that the genetic diversity within *Entamoeba coli* is extensive, and that no less than four genetic subtypes of uninucleate amoebic cysts can be isolated from human faeces. We have also obtained genetic data from protozoan genera that have not been sequenced before.
Conclusion: If future platforms for the diagnosis of intestinal protists are to rely on PCR, comprehensive data from molecular characterisation of these organisms are needed to design, evaluate, validate and optimise PCR protocols.

O48 Synthesis and evaluation of 4-fluoro-amodiaquine a novel antimalarial drug against sensitive and resistant strains of *Plasmodium falciparum*

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Objectives: Resistance to chloroquine (CQ) in *Plasmodium falciparum* malaria has become a major health concern of the developing world. This resistance has prompted a re-examination of the pharmacology of alternative antimalarials that may be effective against resistant strains. Amodiaquine (AQ) is a 4-aminoquinoline antimalarial which is effective against many chloroquine-resistant strains of *P. falciparum.* However, clinical use of AQ has been severely restricted because of associations with hepatotoxicity and agranulocytosis. Based on a knowledge of the metabolic basis of amodiaquine toxicity, the aim of this study was to examine the effects of replacing the 4′OH function of amodiaquine with fluorine.
Materials and Methods: A successful four-step synthesis of a new series of 4′-fluoro analogues has been designed and applied to the synthesis of an array of 10 analogues. Malaria parasites were maintained in continuous culture using the method of Jensen and Trager. Antimalarial activity was assessed with an adaption of the 48-h sensitivity assay of Desjardins et al.. using [3H]-hypoxanthine incorporation as an assessment of parasite growth.
Results: The chemistry in the 4′fluoro series provided the target compounds in higher overall yields. Initial testing on both series of analogues was carried out on a chloroquine sensitive (3D7) and resistant strains TM6, K1, TM4, V1S and J164 at the Liverpool School of Tropical. It is apparent that several analogues have potent antimalarial activity against sensitive 3D7 strain of the parasite. The data indicates that 6 h is superior to the pyrollidino analogue 6b against all of the strains examined. It is also clear that N-tert butyl analogue 6b is potent against chloroquine resistant strains, though it is not quite as active as amodiaquine (AQ) against both chloroquine senstitive and resistant parasites.
Conclusion: In summary, work conducted in this study has identified several potent back-up compounds to the clinical candidate. It is clear that the 4′fluoro series has several members with potent activity compared to amodiaquine. It has been shown that (6 h) has is slightly less potent than amodiaquine, chloroquine and the clinical candidate (4b). Further studies on the metabolism and pharmacokinetics of 6 h are necessary.

O49 Antimicrobial resistance in *Salmonella* infections associated with foreign travel

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Objectives: Non-typhoidal *Salmonella* (NTS) causes gastroenteritis and can lead to serious invasive illness and death. Many cases of NTS go untreated as they cause a mild illness. In more serious cases treatment is required and the fluoroquinolones are often the drug of choice. Isolates with decreased susceptibility to ciprofloxacin (DCS), which is usually associated with resistance to nalidixic acid (NA), are on the increase. It has been suggested that foreign travel is a risk factor for the acquisition of *Salmonella enterica* isolates with NA resistance and DCS. This study aims to investigate the link between foreign travel and NA resistant/DCS isolates in Liverpool.
Methods: NTS strains from 2003 onwards were characterised for resistance to NA and DCS. A database containing all *Salmonella* strains detected by the Microbiology Service at a Liverpool Teaching Hospital from 2003 was merged with statutory notification of diseases data that contains the travel history (validated through an enhanced questionnaire) to investigate the link between NA resistance, DCS and foreign travel.
Results: 433 unique NTS strains were isolated. NA susceptibility DCS information was available for 371 (85.6%) and 364 (84.1%) of cases respectively. NA resistance was found in 82 (22.1%) and DCS in 84/364 (23.1%) of the isolates. Approximately one fifth of cases of salmonellosis had travelled abroad during the incubation period. Significantly higher levels of NA resistance (travel 38.4% vs no travel 18.1%; RR 2.12, 95% CI 1.45–3.09) and DCS (travel 37.5% vs no travel 19.5%; RR 1.92, 95% CI 1.32–2.81) were found in isolates associated with foreign travel.
Conclusions: Foreign travel is a significant risk factor for the acquisition of *Salmonella* isolates with NA resistance and DCS. Antibiotic resistance can lead to delays in effective treatment and result in complications. Public health authorities need to engage with the travel industry in order to improve surveillance and prevention of travel related gastrointestinal infections.

Therapy of multi-drug resistant Gram-negatives

S57 Carbapenem-resistant *Acinetobacter*: a study of class D β-lactamases

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Carbapenem resistant *Acinetobacter* represent a major threat to our antibiotic armamentarium. In general, Class B and D β-lactamases form the basis for this phenotype. Class D β-lactamases are serine enzymes that are either monomers or dimers that possess unique structural motifs. By utilizing a carbamylated Lysine, a distinctive complex hydrogen-bonding network is created to fix the β-lactam in the substrate-binding pocket and assist in binding, acylation and deacylation. As a result of substitutions accelerated by β-lactam use, many class D enzymes emerged that possess functional and structural properties which confer a selective advantage to the bacterium housing the carbapenem hydrolyzing β-lactamase. Important carbapenemases in *Acinetobacter* include: $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24/40}}$, $bla_{\text{OXA-48}}$, $bla_{\text{OXA-58}}$, $bla_{\text{OXA-66}}$. With these notions in mind, our purpose will be to i) review the molecular epidemiology and genetics of the major class D serine carbapenemases that are present in *Acinetobacter* spp; ii) analyze how amino acid changes have altered substrate specificity by using select examples; and iii) highlight changes in the expression of efflux pumps and outer membrane proteins in the amplification of carbapenem resistance. The crystal structures of important class D enzymes will be reviewed and analyzed.

S58 Multidrug-resistant *Pseudomonas aeruginosa*

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In recent decades *Pseudomonas aeruginosa* has emerged as a major threat as a result of the significant mortality associated with pneumonia and bacteraemia, and the evolving resistance exhibited by the pathogen to numerous antibacterials.
Since a timely and appropriate therapy is needed for severe P aeruginosa infections, clinicians should be aware of the risk factors associated with this pathogen and with multidrug resistance. There is still debate if a combination or a monotherapy should be instituted for *Ps. aeruginosa* infections.
The use of combination therapies for *Ps. aeruginosa* pneumonia has been a long-advocated practice, but the potential increased value of

combination therapy over monotherapy remains controversial. However, empirical combination therapy maximizes the chances of bacterial coverage, especially in severe infections, and likely exerts a lower resistance selection pressure.
Upon confirmation of *Ps. aeruginosa* infection, treatment should be given according to the site of infection, the pathophysiology of the patient, the pharmacokinetic/pharmacodynamic profile of the antimicrobials, and the antimicrobial susceptibility pattern, including the MIC values.
For strains that are resistant to all antimicrobials but colistin, this antimicrobial is advocated as the choice option either in mono- or in combination therapy. Indeed, since there are no novel antibiotics in the drug development pipeline for multidrug-resistant *Ps. aeruginosa*, old antibiotics, such as the polymyxins (ie, colistin, also known as polymyxin E and polymyxin B), have re-emerged as the last resort therapy. However, current clinical use of colistin is largely informed by inadequate and, in some cases inaccurate, pharmacokinetic and pharmacodynamic data.
Rifampin, sulbactam, carbapenems, fosfomycin are all possible options for combination treatment, due to their *in vitro* synergistic effect. Among carbapenems, doripenem seems to possess a lower potential for resistance selection and a more favourable pharmacokinetic profile. Newer anti-pseudomonal antibacterials are expected to be available in the near future. Among them, experimental polypeptides (i.e. the anti-PcrV immunoglobulin G antibody) may provide a new therapeutic approach.

S59 ESBL-producing enterobacteria beyond carbapenems

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Carbapenems are considered the drugs of choice for the treatment of serious infections caused by ESBL-producing enterobacteria. However, alternatives are needed because carbapenem-resistance due to carbapenemases and other mechanisms are increasing worldwide. For the treatment of uncomplicated cystitis due to ESBL producers, observational studies showed high cure rates with oral fosfomycin, nitrofurantoine, and amoxicillin–clavulatane when a susceptible isolate was involved. Mecillinam, which is stable against ESBLs, is also a potential option. For patients with other types of infections including complicated urinary tract infections, the options are more limited. Some isolates may show low MIC to specific cephalosporins depending on the type of ESBL produced; PK/PD data suggest that these infections can be treated with these cephalosporins (particularly cefepime) using appropriate doses, but this practice is not recommended for empirical therapy because the MIC cannot be predicted. Adding an aminoglycoside to a standard regimen is a reasonable option for selected patients at low risk of renal toxicity in areas where prevalence of aminoglycoside resistance among ESBL-producers is low. The efficacy of combinations of cephalosporins with β-lactamase inhibitors (as cefoperazone-sulbactam, or as the combination of a cephalosporin and amoxicillin/clavulatane) and temocillin are to be studied in different clinical settings. However, the worldwide spread of the multi-drug resistant clones of *E. coli* and *K. pneumoniae* further reduce the therapeutic options, for which only carbapenems, tigecycline and colistin might be active. While older drugs, such as fosfomycin (probably in combination), merit being investigated in systemic infections, new drugs active against these organisms are clearly needed. The increasing prevalence of ESBL producing Enterobacteria in the community and hospital is not just a threat anymore but a real everyday problem.

S60 Carbapenemase-producing enterobacteria

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Resistance to carbapenems due to the production of metallo-β-lactamases (MBL) or KPC enzymes in Enterobacteriaceae is an increasing international public health problem. An MDR or even an PDR phenotype is associated with carbapenem resistance in Enterobacteriaceae, because these strains usually harbor mechanisms of resistance to aminoglycosides and quinolones, as well. Nevertheless, some of these strains often exhibit low-level resistance to carbapenems with MICs remaining in the susceptible range rendering the therapeutic role of carbapenems questionable.
The armamentarium against PDR Gram-negative microorganisms has almost been exhausted. The only options left are colistin, an antibiotic introduced in the 1950s, and tigecycline, a modified minocycline. Monotherapy or combination therapy with colistin is most often used but recently, the emergence of colistin-resistant strains of *Klebsiella pneumoniae* has limited our therapeutic options even further. There is accumulated evidence on the *in vivo* activity of tigecycline against MDR Enterobacteriaceae but the low levels of the drug achieved in blood indicate the necessity of a higher dose in case of bacteraemia. Some of the MBL or KPC producing Enterobacteriae are *in vitro* susceptible to fosfomycin. Clinical experience in the setting of serious infections by carbapenemase-producing bacteria is still limited. Finally, combination regimens have very often been used empirically in clinical practice although scientific evidence on the advantages of combinations is usually scarce with the exception of carbapenem-susceptible *K. pneumoniae,* for which clinical data suggest that the combination of meropenem and gentamicin could be active *in vivo*, in the case of meropenem MIC $\leqslant 2$ μg/ml.
The emergence of carbapenemase-producing Enterobacteriaceae highlights the "end of antibiotics". Concentrated efforts are needed to preserve and wisely use the few options available. In the meantime, intensified infection control measures may protect our hospitalized patients from these difficult to treat pathogens.

New horizons in the diagnosis of bacterial sepsis

S62 Biomarkers for the diagnosis of sepsis: do they provide added value?

B. Müller (Aarau, CH)*

The ambiguities of clinical signs and the limitations of current microbial techniques for the diagnosis of bacterial infections – and to grade their severity – are well known. The use of biomarkers provides a novel, complementary approach to diagnose infection, and to estimate treatment response and the outcome of patients. A plethora of proteins has been speculated to be "promising markers" in observational studies including c-reactive protein, various interleukins and chemokines and procalctionin, amongh others. Unfortunately, in most infections including sepsis, a true "gold standard" for diagnosis does not exist. Thus, the true "added value" of a biomarker in clinical use can only be assessed in interventions studies, where the endpoints are safety and antibiotic use. In the hospital setting, this has only been shown for procalcitonin (PCT) in multiple randomized controlled interenvetion studies from several independent groups. With a similar outcome antibiotic exposure could be reduced from by 50 to 75%. For prognostic assessment, other biomarkers (e.g. adrenomedullin) have demonstrated high predictive potential to estimate the risk for mortality in the short and long term, and other adverse outcomes. A critical appraisal of the advantages and limitations of biomarkers in different clinical situations is mandatory. We discuss the current data on the use of PCT and other biomarkers for the diagnosis, treatment guidance, and prognostic assessment of bacterial infections, and their potential role in the overall assessment of patients with sepsis and respiratory tract infection as its most important precursor.

S63 Rapid detection of pathogens in sepsis: molecular techniques versus culture

C. Vandenbroucke-Grauls (Amsterdam, NL)*

Molecular techniques are gaining more and more interest for rapid diagnosis of severe bacterial infections. DNA-hybridisation probes and PCR-based detection are used for rapid identification of bacteria after

the first signal of growth in conventional blood cultures. PCR-assays can also be used for direct detection of pathogens in blood. Such PCRs can be aimed at specific pathogens, or be more broad-range, in which case the PCR is followed by sequencing of the PCR product for identification. PCR can also be used for quantification of the amount of bacterial DNA in blood; several studies point to the value of the bacterial DNA load (BDL) in blood as a marker of severity of infection. This has been shown for pneumococci and meningococci in particular, and there is anecdotal evidence that BDL also correlates with severity of infection in staphylococcal infections. Interpretation of the results of PCR applied directly on blood samples needs further study, however, because several aspects of the kinetics of the presence of bacterial DNA in blood during infection are still unknown. In particular, we must be aware that it is difficult to define the best gold standard for bacteraemia, that PCR detects DNA, rather than living pathogens, that there is always a risk of contamination, and that little is known yet about background bacterial DNA in blood.

S64 Significance of DNAemia in sepsis

K.P. Hunfeld (Frankfurt/M., DE)*

Sepsis is associated with mortality rates ranging from 20% to 50% and represents the second leading cause of death in the non-coronary intensive care unit. Early diagnosis of sepsis followed by prompt appropriate treatment improves the prognosis of septic patients. It has been suggested that nucleic-acid-based technology such as PCR is more sensitive and can also shorten the time to result when compared with conventional blood culture techniques. To date, however, little is known about the kinetics and the clinical and therapeutic relevance of bacterial DNA present in the blood of patients with sepsis over time in the course of an invasive blood stream infection. The recent introduction of more standardized new PCR-based diagnostic assays including tests that provide a more exact quantitative measurement of DNA, therefore, may open a window of opportunity for a better understanding of the kinetics and clinical significance of microbial DNA circulating in the blood of patients during a blood stream infection. In fact, the potential influence of bacterial DNA on the severity and outcome of blood stream infections is underlined by the findings of recent clinical and laboratory studies suggesting that procalcitonin plasma levels and SOFA scores were significantly higher, length of ICU and hospital stay were longer, and survival was clearly decreased in subgroups of septic patients revealing positive findings in both PCR testing and blood culture. Whether a better insight into the clinical significance of DNAemia will indeed contribute to more rapid initiation of better-tailored therapy and improved management of septic patients in conjunction with other laboratory markers, however, awaits further evaluation of in laboratory studies and interventional clinical trials. The present lecture will summarise what is known from recent studies on the diagnostic and clinical significance of DNAemia in patients with sepsis.

Diagnostic dilemmas – things that look like infections but aren't

S65 Non-infectious causes of fever in the critical-care unit

M. Skalweit (Cleveland, US)*

"Fever" is a common symptom in critically ill patients and often sets into play a series of investigations by health care providers to establish an etiology. Recent practice guidelines for the evaluation of fever in the ICU patient recognize that "knee-jerk" responses to this syndrome, in the absence of careful clinical evaluation, lead to increased costs and morbidity for patients. In addition, many diagnostic studies are uninformative. Nevertheless, non-infectious causes of fever are often diagnoses of exclusion; a balance between expensive and invasive investigations, and "old fashioned" clinical acumen needs to be applied to evaluate fever in the ICU. In this session, common non-infectious etiologies of fever will be discussed with illustrative case examples, e.g. gout, drug fever, vasculitis, etc. In addition the role of molecular tests that are used to assist in determining the infectious etiologies of fever (PCR methods and biomarkers such procalcitonin and 1,3-β-glucan) will be reviewed.

S67 When are arthritis real infections?

M.A. Muniain (Seville, ES)*

Joint diseases may present as acute, chronic and at the same time as monarticular or polyarticular arthritis. Most of the aetiological agents who have been identified in monarticular infections may also cause polyarticular infections. Similarly, most of the polyarticular non infectious arthritis may appear as monarticular. In many clinical situations extra-articular manifestations may differentiate infectious from non infectious arthritis.

The recent development of new technology for culture and molecular biology has permitted to recognise virus or bacteria into the joints of degenerative, inflammatory and of course, in infectious arthritis. Reactive arthritis (ReA) is the conceptual ground somewhere between septic arthritis and autoimmune diseases such as rheumatoid arthritis. The fact that some arthritis (Whipple, SAPHO etc) now have been recognised as infectious arthritis and may respond to antibiotic treatment, as prompted to treat a number of patients with arthritis of "unknown aetiology" with antimicrobials.

Form a practical point of view the answer to the question "When are arthritis real infections?" may be "When we can treat and cure the infection". Three aetiologies are involved in most or the patients with acute monarticular arthritis; mechanical, infections and microcrystallines. Inflammatory arthritis may also present as monarticular arthritis. But besides that, more than 20 different non infectious diseases can produce acute arthritis ("pseudoseptic" arthritis").

The tools for the diagnosis of arthritis are limited. As a matter of fact in Rheumatology the "pattern" of the disease is particularly important; what joints are involved, what is the sequence, the accompanying symptoms or even the duration of the disease. There are no definitive laboratory markers (except crystals and cultures in the synovial fluid). Biochemical and cytology values in synovial fluid permit to classified arthritis as mechanical, inflammatory or septic, but in a particular patient they have only a marginal value. In critical reviews for cytology in the synovial, fluid the evidence is mainly anecdotal and there are no reports of specifity, sensitivity and reliability.

In this topic as in many other, medical history, epidemiological investigation and clinical examination are the main stone for the diagnosis. Detailed history and physical examinations may raise the possibility of some initially unsuspected systemic or localized non infectious disease.

S68 Autoimmunity and infection: a bidirectional relationship

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The hypothesis of an infectious origin for autoimmune diseases has received great attention during the recent years. Microbial agents or viruses can induce autoimmune diseases by a variety of mechanisms. For example, proteins of certain infectious agents can act as polyclonal activators on unique lymphocyte subsets. Viruses can preferentially infect/destroy a particular T cell subset, leading to an imbalance in the immune response. In other instances, infectious agents can up-regulate Th1 cytokines, thereby increasing selected expression of molecules such as major histocompatibility complex (MHC) glycoproteins, as well as activation of costimulatory molecules. Several microbial agents have been found to encode superantigens that can selectively activate subsets of T cells. Microbes can also direct the release of cytokines and chemokines, which can act as growth, differentiation, or chemotactic factors for different Th populations and regulate expression of MHC class I and class II molecules.

On the other hand, the healthy immune system is tolerant to the molecules of which the body is composed of. However, one can find

that among the major antigens recognized during a wide variety of bacterial viral and parasitic diseases, many belong to conserved protein families, sharing extensive sequence identity or conformational fits, with host's molecules, namely molecular mimicry. Antigenic similarity of either molecules' linear amino-acid sequences or their conformational structure between antigens of infectious agents and host tissues might trigger an immune response against the shared determinant. As a result, the tolerance to autoantigens breaks down, and the pathogen-specific immune response that is generated cross-react with host structures to cause tissue damage and disease.
In this presentation, the cases of Sjögren's syndrome, systemic lupus erythematosus and the antiphospholipid syndrome, among others, will be reviewed as clear examples of autoimmune diseases where an infectious origin is postulated.

Pneumococcal infection: insights into pathogenesis and therapeutic potential

S69 The interface between innate and adaptive immune responses

D.H. Dockrell (Sheffield, UK)*

Nasopharyngal colonisation with pneumococci is frequent but in contrast pneumococcal pneumonia and invasive pneumococcal disease are comparatively infrequent events. This reflects the success of both innate and adaptive host responses to pneumococci. The presence of a variety of virulence factors including polysaccharide capsule and pneumolysin challenges the host response. Epithelial cells sense microbial products and release cytokines in response to pneumococci in the lower respiratory tract. Soluble factors, including complement factors, activated through both the classical and alternative pathway, play an important contribution to host defence in the lung. Alveolar macrophages play a critical role as the resident phagocytes in the lung clearing bacteria from the lung and orchestrating the inflammatory response. Once resident defence becomes compromised an inflammatory response including the recruitment of neutrophils becomes essential to microbial control but comes at the potential cost of compromising lung homeostasis. The regulation of this process also requires macrophage competence to ensure a limited inflammatory response which results in bacterial clearance but without lung injury. During the evolution of pulmonary infection a number of critical transition points occur when specific molecules and host responses are critical in determining how the infection evolves and what the outcome of infection will be. The infectious inoculum, the virulence of the pathogen and host susceptibility determines the position of these transitions and the relative role of key host defence strategies. There is increasing recognition of the role of T-cells, B-cells and dendritic cells in the pulmonary response to pneumococci. The integration of these various host responses to pneumococci, in most cases, represents a paradigm which provides a template for successful host responses to pathogenic pulmonary bacteria.

S71 Bacterial evolution in the face of immunological pressure

C. Donati, A. Muzzi, A. Covacci, R. Rappuoli, V. Masignani, M. Barocchi (Siena, IT)*

S. pneumoniae is part of the normal upper respiratory tract flora but it can become pathogenic causing a variety of diseases, which range from otitis media and sinusitis to pneumonia, septicemia, and meningitis. Due to its intimate relationship with the human host, *S. pneumoniae* has evolved a series of strategies to vary its genetic repertoire to evade the host immune response. Using their ability to recombine DNA acquired from the environment, *S. pneumoniae* strains are able to renew their dispensable genome (i.e. those regions of the genome that are not shared by all isolates). Dispensable genes are frequently acquired and lost, causing a loss of correlation between the phylogenetic history of strains and the presence of genes encoding proteins with antigenic properties. As a consequence these organisms have access to a genetic repertoire – the pan-genome – that is larger than the genome of any component strain. At the species level the pan-genome of *S. pneumoniae* grows as the number of sequenced strains increases due to the influx of genetic material from closely related species, and the mode of this growth positions the pneumococcal species on the edge between open and closed pan-genome. A second strategy for antigenic variation is shown by the evolution of pili, long filamentous appendages involved in adhesion to host cells. Pili are encoded in the rlrA islet, a 12 kb genomic region, that consists of the rlrA transcriptional regulator, rrgA, rrgB and rrgC, coding for LPXTG proteins forming the structure of the pilus, and srtB, srtC and srtD, coding for sortase enzymes catalyzing the pilus polymerization reaction. Due to their exposure to the host immune response, rrgA and rrgB are under positive selection that causes an increased rate of fixation of new alleles, and exist in three distinct clades that correlate with the MLST designation of the strains. Instead the rrgC, srtB, srtC and srtD genes, coding for proteins not directly exposed to the host immune system, are not under positive selection. In the region containing these genes, there is evidence of homologous recombination, and 4 major recombination hotspots can be identified. Due to the homogenizing effect of recombination these genes, differently from the major structural components of the pilus, are well conserved at the sequence level. In addition, piliated strains of Pneumococcus have been associated with multi-drug resistance cassets that may provide *S. pneumoniae* added fitness.

S72 Pneumococcal vaccination

D. Bogaert (Utrecht, NL)*

Streptococcus pneumoniae is worldwide a leading cause of morbidity and mortality due to respiratory and invasive diseases. WHO estimates that annually at least one million children under 5 years of age die of infections caused by this pathogen. The more than 90 different capsular serotypes make it difficult to design a preventive strategy with universal pneumococcal coverage. In 1983, a 23-valent polysaccharide vaccine was marketed, being effective against invasive pneumococcal bloodstream infections in adults. Unfortunately, this vaccine proved low immunogenic in children due to it's T-cell independent type of immune-protection. In 2000, a new 7-valent conjugate vaccine (PCV7) was licensed in the USA, protecting very well against invasive pneumococcal diseases (IPD) caused by vaccine serotypes, also in infants and young children. However, within the years following implementation of PCV7, in addition to a serious drop in vaccine serotype diseases, a significant increase in IPD caused by non-vaccine serotypes was observed. The impact of this so called 'replacement' disease on the total incidence of IPD seems to vary by population and country. Recently, two extended pneumococcal conjugate vaccines have been approved for use in children and adults, covering most of the currently 'emerging serotypes'.
In this presentation, the following topics will be addressed; What most likely causes 'replacement' disease: vaccine pressure, 'unmasking' of non-vaccine serotypes, secular trends or a combination of those? Do we expect the new extended conjugate vaccines to solve the issue of 'replacement' disease? Do we expect in addition to shifts within IPD causing serotypes also shifts in diseases caused by other respiratory bacteria? What are potential new vaccine candidates, and what do we expect of those vaccines with respect to impact on pneumococcal and non-pneumococcal diseases?

Nosocomial infections in critical-care patients

S73 Ventilator-associated tracheobronchitis

S. Nseir (Lille, FR)*

Ventilator-associated tracheobronchitis (VAT) is an intermediate process between colonization of lower respiratory tract and ventilator-associated pneumonia (VAP). Postmortem studies showed a continuum between

bronchitis and pneumonia in mechanically ventilated ICU patients. The progression from colonization to VAT, and in some cases to VAP depends on quantity and virulence of the bacterial pathogen, and host lung defenses.
VAT is common in mechanically ventilated patients. Its incidence ranges from 3–10% of ICU patients. Definition of VAT is matter of debate. Our group defined VAT using all the following criteria: fever (>38ºC) with no other recognizable cause, purulent sputum production, positive culture of respiratory specimen at significant threshold, and no radiographic signs of new pneumonia. Portable chest radiograph is inaccurate in diagnosing new infiltrates in mechanically ventilated patients. Therefore, differentiating VAT from VAP could be a difficult task in ICU patients. VAT is frequently caused by Gram-negative bacilli, especially *Pseudomonas aeruginosa*. Although several studies investigated risk factors for VAP, few have evaluated risk factors for VAT. However, risk factors for these infections appear to be similar. Age >60 years, COPD, prior antimicrobial treatment and surgery were identified as risk factors for VAT.
Tracheobronchitis is characterized by lower respiratory tract inflammation and increased sputum production. These factors may generate weaning difficulties, resulting in longer duration of mechanical ventilation. In a large cohort of mechanically ventilated patients, VAT was significantly associated with longer duration of mechanical ventilation and ICU stay.
Beneficial effects of antimicrobial therapy were recently reported in VAT patients. In a randomized blinded placebo-controlled trial, aerosolized antibiotics significantly reduced the incidence of subsequent VAP. Further, aerosolized antibiotics increased weaning from mechanical ventilation, reduced usage of systemic antibiotics and antibiotic resistance. The impact of systemic antibiotics on outcomes of VAT patients was evaluated in a randomized unblinded controlled study. Antibiotic treatment increased mechanical ventilation free days, and reduced the incidence of subsequent VAP and ICU-mortality. Future studies should confirm these promising results, and determine the best duration of antimicrobial therapy in VAT patients.

S74 CMV and HSV in critical-care patients: pathogens or bystanders?

C. Linssen (Maastricht, NL)*

Cytomegalovirus (CMV) and human herpes simplex virus (HSV) belong to the family of Herpesviridae. Both CMV and HSV are highly prevalent and ubiquitously distributed. In the immunocompetent adult host CMV and HSV infections usually have a benign course. As is the case with other herpesviruses, the initial infection is followed by a lifelong latent infection from which reactivation can occur. In immunocompetent individuals, asymptomatic viral shedding may be detectable in respiratory materials such as saliva or sputum in case of both HSV and CMV and also in urine in case of CMV. This viral shedding in patients without active viral disease makes it difficult to diagnose active disease in patients, especially since the symptoms of CMV and HSV infection (excluding typical skin lesions) are often non-specific. In immunocompromised patients CMV can lead to severe disease varying from retinitis and pneumonitis to generalized CMV disease. Occasionally, HSV may cause pneumonia as a result of immunosuppression, with a high mortality. However, critically ill patients admitted to the intensive care unit (ICU) are considered immunocompetent. Studies performed in ICU patients have shown these patients to be at risk for severe infections with CMV and HSV. In case of CMV, results from previously conducted studies point towards reactivation from latency as the most likely explanation rather than primary infection in ICU patients. Recent studies suggest that active CMV infection is quite common in ICU patients, with a prevalence up to 35%, depending on the sub-group of patients studied. Furthermore, an association between the presence of a CMV infection and increased mortality in critically ill patients was found. In recent years, the interest in HSV as a causative micro-organism in ICU patients has increased, especially as a cause of pulmonary infection. Recent reports detected HSV-1 in respiratory samples from ICU patients not considered to be primarily immunocompromised. In all studies a significant adverse effect of HSV-1 shedding in the respiratory tract on clinical outcome was established. Two studies showed an association between a high load of HSV present in the respiratory tract and increased mortality. At this moment it is not clear whether the association of both CMV and HSV with increased mortality is due to the micro-organism itself or that it is just an indication of the deteriorating physical condition of the patients leading to reactivation of the virus.

S75 The concept of clinical sepsis – when all cultures are negative in a "septic" patient

P. Eggimann (Lausanne, CH)*

Despite all attempts to prevent them, nosocomial infection (NI) may complicate the stay of up to one third of patients requiring ICU management and the development of a sepsis of any severity should be considered as a potential NI. Prompt initiation of broad-spectrum antibiotics and source control are key factor for favorable outcome. A majority of them are device-related and a systematic workshop should be performed to identify them. Documentation of the source of infection may then allow to more specific measures, such as abscess drainage or catheter removal. Guidelines nowadays also include recommendations for systematic periodical revaluation of the evolution in order to adapt antibiotics and other therapeutic measures. In the absence of positive cultures, combination of clinical evolution and some biological parameters may contribute to safe de-escalation strategy.

Bacterial pathogenesis

O77 Role of biotic interactions in microbial adaptation

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During millions of years "arms-race" shaped evolution not only between the species across the planet but also inside the human body. Innate immune system is constantly evolving different strategies to detect and destroy intrusive microbes, while microbes evolving herewith. What are the strategies that microbes can develop to avoid and circumvent immune recognition?
We study the evolution of the model organism *Escherichia coli* under the selective pressure of bacterial killing cells, the macrophages (cell line RAW 264.7), which form an important component of the innate immune system.
In approximately 100 generations all bacterial lines, evolved in the presence of the macrophages, showed a remarkable polymorphism, which did not occur in the controls. The relative abundance of the new morphs fluctuated stochastically over time, exhibiting a mixture of local peaks and short periods of apparent stable frequency. The distinct genotypes are marked by morphological differences clearly seen in the *E. coli* forming colonies and in FACs measurements. Furthermore, the first observed morphs that persist better inside the macrophages because of the slow growth rate and deficiency in metabolism have the same characteristics as SCVs (small colony variants) sampled from patients with various recurrent and persistent infections. Other morphs became resistant to engulfment and possibly killing by phagocytes and showed mucoid phenotype.
Investigating the long-term consequences of biotic interactions is clearly an emerging field of research because has implications on applied biomedicine furthermore it will be able to generate predictions concerning the nature of adaptations of microorganisms to multiple infections and to the immune system.

O78 Intracellular lifestyle of *Streptococcus pyogenes* in human macrophages: survival strategies, replication, egress and re-infection

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Objectives: Our recent studies using tissue biopsies from patients suffering from sepsis, necrotising faciitis and toxic shock demonstrated that *S. pyogenes* survive intracellularly in macrophages and contributes to bacterial persistence during acute deep tissue infections. This study aimed to elucidate the mechanisms by which *S. pyogenes* enters and survives in macrophages during severe streptococcal infections.
Methods: Primary human monocyte-derived macrophages were infected in kinetic experiments with a clinical M1T1 *S. pyogenes* isolate expressing green fluorescent protein (GFP), or an isogenic mutant deficient in M1 protein. Viability of bacteria was assessed by GFP-expression and confirmed by use of a bacterial viability staining kit or viable counts. Uptake, intracellular trafficking, and egress were analysed using flow cytometry, confocal and electron microscopy (EM). Re-infection was assessed by co-culture with FarRed labelled cells.
Results: *S. pyogenes* are taken up by large pseudopod loops in an actin-dependent manner, after which they reside within membrane surrounded vesicles. In kinetic studies, transient co-localisation is seen with early endosomal marker (EEA-1), whereas no association is noted with the late endosomes/lysosomes (LAMP-1 and lysotracker stain). In contrast, infection with the M1 mutant results in fusion with vesicles and significantly reduced bacterial counts intracellularly, as compared to the wild type strain ($p = 0.03$). Moreover, EM-studies demonstrated that the bacteria replicate intracellularly, and that after 6–10 h the bacteria migrate out of the cells. This is an active process as fixed bacteria used in control experiments remains intracellularly. Lactate dehydrogenase (LDH)-release experiments indicate that the egress is associated with cell death.
Conclusions: The findings demonstrate that M1 protein is crucial for intracellular survival of *S. pyogenes* in macrophages. Survival seems to be associated with impaired fusion with the lysosomes. Subsequent intracellular bacterial replication and egress of the bacteria is followed by re-infection of surrounding cells. This is likely an important event for dissemination and progression of severe streptococcal infections.

O79 Inhibition of the inflammasome is a component of immunoparalysis in Gram-negative sepsis

E.J. Giamarellos-Bourboulis, F.L. van de Veerdonk, M. Mouktaroudi, M. Raftogiannis, A. Antonopoulou, M. Georgitsi, J. van der Meer, M.G. Netea (Athens, GR; Nijmegen, NL)*

Objective: Immunoparalysis in sepsis is characterized by impairment of cytokine production by monocytes. Interleukin (IL)-1β to be produced requires cleavage of its pro-form that is mediated through the inflammasome. The function of inflammasome in sepsis was assessed.
Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from 34 healthy volunteers and from 92 patients with sepsis (49 uncomplicated sepsis; 26 severe sepsis; and 16 with septic shock defined by ACCP/SCCM 1992 criteria) caused by Gram-negative bacteria. PBMCs were stimulated with a variety of stimuli in the absence and presence of caspase-1 inhibitor and of crystals of monosodium urate (MSU) that is an agonist of NALP3 inflammasome. IL-1β was estimated by an enzyme immunoassay. Gene transcripts of IL-1β were estimated by RT-PCR.
Results: Release of IL-1β from PBMCs of septic patients was significantly reduced after stimulation with LPS and heat-inactivated isolates (Figure 1a, asterisks denote significant differences with controls). Gene transcripts of IL-1β were lower compared with healthy controls but this difference was not significant. Effect of caspase-1 inhibitor on release of IL-1β is shown in Figure 1b (asterisk denotes significant differences in the absence of inhibitor). Stimulation with MSU yielded significant releases of IL-1β from PBMCs of healthy controls but not of patients with sepsis (Figure 1c). LPS and MSU acted in synergy for the production of IL-1β by PBMCs of healthy volunteers but not of patients with sepsis (Figure 1d; asterisk denotes significant difference by the LPS and MSU compared with single LPS).
Conclusions: Inhibition of the inflammasome is a considerable component of the phenomenon of immunoparalysis presented among patients with Gram-negative sepsis.

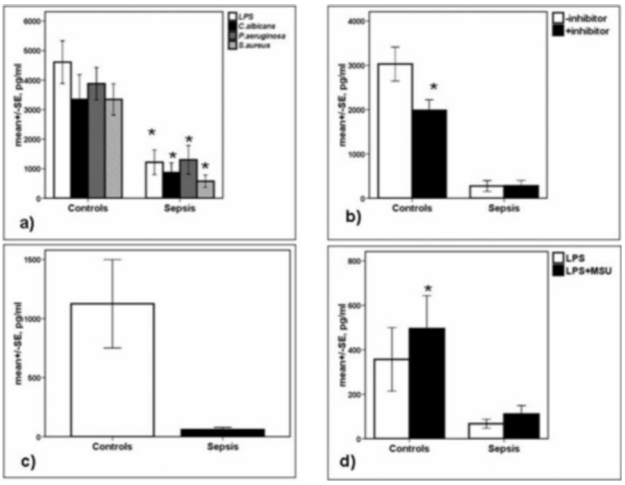

Figure 1.

O80 Fluoroquinolone-resistant *Salmonella Typhimurium* has decreased intracellular survival in macrophages

A. Dehn Lunn, A. Fàbrega, J. Vila, R. Read (Sheffield, UK; Barcelona, ES)*

Objectives: Previous studies have suggested that fluoroquinolone resistance may be associated with attenuated virulence, and this may be the reason for the low levels of fluoroquinolone resistance compared with nalidixic acid resistance in *Salmonella* clinical isolates. We compared the intracellular survival of wild-type (ciprofloxacin MIC: 0.012 ug ml-1), fluoroquinolone-resistant (ciprofloxacin MIC: 64 ug ml-1) and reverted (ciprofloxacin MIC: 1.5 ug ml-1) S. Typhimurium in murine macrophages.
Methods: The fluoroquinolone-resistant S. Typhimurium was obtained *in vitro* by serial passage of the wild-type strain in increasing concentrations of ciprofloxacin, whereas the reverted strain was obtained from passage of the fluoroquinolone-resistant strain on antibiotic-free media. J774.2 macrophages were seeded at 2×10^5 cells per well and left to adhere for 24 hours. Control wells were fixed with paraformaldehyde. Prior to infection, wells were blocked with BSA for 30 minutes. Bacterial cultures were grown to log phase and then diluted to infect the macrophages with an MOI of 100. A gentamicin protection assay was performed, with viable counts performed at 0, 1, 4 and 24 hours.
Results: Fluoroquinolone-resistant S. Typhimurium showed significantly decreased overall survival in comparison to wild-type (median area under the curve (AUC) of 1.49×10^6 versus 1.43×10^5, statistically significant at the 1% level). In most experiments, there was a log difference in bacterial concentration (cfu ml-1) between the wild-type and resistant strains at all timepoints. There was not a significant difference between the wild-type and reverted strains or between the reverted and fluoroquinolone-resistant strains. To determine whether there was a difference in intracellular replication, data were analysed as a percentage of the bacterial concentration at $t = 0$ (that is, 90 minutes after infection). There was not a statistically significant difference between groups ($p = 0.96$).
Conclusions: Fluoroquinolone-resistant S. Typhimurium showed decreased survival in murine macrophages. Further work is needed to identify the genes responsible for this change, since the mutants were not isogenic.

O81 Binding and activation of plasminogen on *Fusobacterium necrophorum*

P. Kuusela, N. Friberg, H. Jarva, P. Mattila, R. Soliymani, M. Baumann (Helsinki, FI)*

Objective: Many bacteria have plasminogen (Plg) receptors (PlgR) on their surface. Binding to PlgRs enhances activation of the bound Plg to active enzyme plasmin and leads to protection of surface-associated plasmin against physiological plasmin inhibitors. Surface-associated plasmin is thought to play a pathogenetic role in various infections. In this work we have studied Plg binding and activation on *Fusobacterium necrophorum* (Fnec), an important Gram-negative rod-shaped anaerobe causing Lemierre's syndrome characterized by sepsis and internal jugular vein thrombosis preceded by an oropharyngeal infection.
Methods: Plg binding was monitored by incubating bacteria isolated from clinical samples with I125 Plg alone or in various combinations with lysine analog epsilon-amino-caproic acid (EACA), tissue-type plasminogen activator (tPA) and alfa2-antiplasmin (alfa2AP), the physiological plasmin inhibitor, followed by counting of the bound radioactivity or by analyzing the bound Plg with SDS-PAGE and autoradiography. Plg activation was recorded by following the breakdown of the chromogenic substrate S-2251 in various reagent combinations. Tentative fusobacterial PlgRs were identified by means of ligand blotting of bacterial outer membrane proteins with Plg and by subsequent MALDI-Tof analysis.
Results: Fnec (n = 11) bound significantly better iodinated Plg than *F. nucleatum* (n = 14). Binding was inhibited by EACA and lead to enhanced activation by tPA. Formed plasmin was protected against inhibition by alfa2AP. Additionally, SDS-PAGE analysis of the bound plasmin revealed trimming of the formed Glu-plasmin to a slightly shorter Lys-form of the enzyme. Ligand blotting/MALDI-Tof analyses revealed that acyl-CoA dehydrogenase was a predominant Plg binder among the fusobacterial outer membrane proteins.
Conclusions: The present results show that Fnec has a much stronger capacity to bind Plg than *F. nucleatum.* Inhibition of binding by EACA indicates that lysine binding sites in the N-terminal portion of Plg are involved in the binding. Inability of alfa2AP to inhibit formed plasmin activity emphasizes the receptor association of plasmin. Trimming of the surface-associated Glu-plasmin into the Lys-form speaks for an increased affinity between plasmin and PlgRs. Acyl-CoA dehydrogenase is a strong tentative PlgR on Fnec. The data indicate that formation of surface associated plasmin on Fnec may play an important role in tissue invasion and in escape from thrombi.

O82 Characterization of the *Klebsiella oxytoca* cytotoxin

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Objectives: *Klebsiella oxytoca* has been shown to be the causative agent for Antibiotic Associated Hemorrhagic colitis (AAHC). The disease occurs during medical treatment with penicillin and results in a sudden onset of bloody diarrhoea and abdominal cramps. Pathological features are mucosal haemorrhage and mucosal oedema that affect the ascending colon and cecum. The histological features of the AAHC resemble toxin-induced colitis and a cytotoxic substance is found in conditioned (cell-free) medium of bacterial cultures.
We aim to identify genes involved in cytotoxin production and secretion. The identification of cytotoxicity related genes will allow the biochemical description of the toxin and should provide insights into the effects on the host cells.
Methods: A human epithelial Hep2 cell line is used to measure cytotoxicity of the *Klebsiella* product qualitatively and quantitatively via the MTT viability assay. A random miniTn5 transposon mutagenesis created a library of randomly inserted knock out mutants in an AAHC patient isolate of *K. oxytoca.* The mutant library was screened for loss of cytotoxicity. The transposon insertion sites of toxin negative mutants were identified through isolation of adjacent chromosomal DNA via a plasposon rescue protocol followed by DNA sequencing. Finally, specific deletion mutants of the identified genes were generated and complementation of the cytotoxin negative phenotype was performed. Structure and regulation of cytotoxin production genes and the cytotoxin's chemical nature are under investigation. Cytotoxin positive and -negative *K. oxytoca* strains will be screened furthermore for distribution of those genes.
Results: So far screening the mutant library revealed two toxin negative mutants. The insertion sites cluster in the same region, indicating an operon. Three functionally related putative genes could be identified: a non-ribosomal peptide synthase, a DAHP synthase and a Xaa proline amino peptidase. The genes are involved in the non-ribosomal peptide biosynthesis and are not conserved in other *Klebsiella* species.
Conclusion: The clustering of mutations leading to a cytotoxin negative phenotype suggests that the non-ribosomal biosynthesis pathway is essential for cytotoxin production. The substance family known to be synthesized through that pathway in other organisms includes numerous fungal and bacterial effector substances, including antibiotics, cytostatics and siderophores.

O83 Infrequent deletion of the chromosomal genes speB and speF in GAS clinical isolates

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Objectives: The chromosomal genes speB and speF do not encode true exotoxins (SpeB is a cysteine protease and SpeF a mitogenic factor), but are commonly included in the exotoxin gene profiling of Group A Streptococci (GAS) and have both been implicated in virulence. The aim of the present work was to confirm the presence of these genes in all GAS isolates and to characterise in detail any putative genomic deletions.
Methods: The genes speA, speB, speC, speF, speH, and ssa were PCR-screened in a total of 724 GAS isolates (160 from invasive infections and 564 from pharyngitis). PCR-negative results for speB and speF were confirmed by Southern blot hybridization with probes specific for each gene. Long-range PCR with primers specific for the regions flanking the putative deletions was performed, and the exact deletion breakpoints were determined by sequencing.
Results: The absence of speB was detected in four isolates, one of which was also the only isolate negative for speF. The latter was an emm28/ST52 strain isolated from blood. Sequencing confirmed a deletion of 4933 bp by comparison with the published genome sequence of the emm28 strain MGAS6180, between nucleotides 2463 and 7395 (GenBank NC007296). The other speB-negative isolates belonged to emm types 4, 11, and 13, and were all isolated from pharyngitis cases.
Conclusion: The presence of the speB and speF genes is a characteristic of GAS recovered from human infections. Still, four isolates (0.6%) were speB-negative and one (0.1%) was speF-negative. The speB– speF– isolate was apparently highly virulent, in spite of the 4933 bp deletion encompassing several genes encoding proteins recognized to be important for virulence, such as SpeB, SpeF, the transcriptional regulator Rgg, the glycerol dehydrogenase, as well as four ORFs of unidentified function.

O84 Lipocalin 2 is detrimental during murine pneumococcal pneumonia

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Objectives: Lipocalin 2 (Lcn2) is an antibacterial protein, known to interfere with bacterial siderophore-dependent iron acquisition. Thus, Lcn2–/– mice are highly susceptible to infections with siderophore-dependent pathogens such as *E. coli* and mycobacteria. Although no siderophores have been detected in *Streptococcus pneumoniae*, infection with this bacterium induces tremendously elevated levels of Lcn2 – and the biological role of this finding remains elusive. We therefore attempted to investigate the role of Lcn2 during pneumococcal pneumonia.

Methods: Age- and sex-matched C57BL/6 and Lcn2–/– mice were inoculated intranasally with *Streptococcus pneumoniae* and sacrificed 6 or 48 hours after infection or monitored for survival. Lungs were homogenized and plated on agar plates for bacterial counts and cytokine detection or embedded in paraffin for histological analysis.
Results: We observed significantly more KC and IL-6 in the bronchoalveolar lavage of Lcn2–/– mice 6 hours after infection. This resulted in significantly increased neutrophil influx after 6 hours leading to improved bacterial clearance after 48 hours. Consistent with this observation we found decreased cytokine levels and reduced lung inflammation in Lcn2–/– mice at later time-points after infection. Finally, Lcn2KO mice displayed a significant survival advantage over wild type animals.
Conclusion: Lcn2 is detrimental during murine pneumococcal pneumonia. We postulate that Lipocalin 2 prevents the early induction of inflammation upon *S. pneumoniae* infection and thus impairs clearance of bacteria and survival.

O85 Apoptosis of neurogenic cells in an *in vitro* model of bacterial meningitis

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Background: Bacterial meningitis (BM) leads to learning deficits in up to 50% of survivors. The histomorphologic correlates of the deficits include apoptosis of neurogenic cells in the hippocampus, a brain structure involved in learning.
Objective: To use an *in vitro* system of hippocampal neuronal differentiation to (i) identify triggers and molecular mechanism of apoptosis and, (ii) to investigate whether the differentiation stage during neurogenesis determines the vulnerability for apoptosis.
Methods: Stem- and neuronal progenitor-cells were isolated from postnatal (P4–6) rat hippocampus. Dissociated cells were driven into neuronal differentiation by the addition of BDNF for 21d. Cells were challenged with different stimuli characteristic for BM: Growth factor deprivation (GFD) by omission of BDNF, EGF and FGF for 24 h, application of TNF-α (20ng/ml) for 24 h and exposure to live bacteria (*Streptococcus pneumoniae*), together with penicillin and streptomycin (10ng/ml) to cause bacteriolysis, for 2 h. Differentiation stage was documented by immunoassaying for nestin (stem cells), doublecortin (immature neurons) and MAP2 (neurons). Neuronal differentiation *in vitro* was documented by a shift in the predominant staining pattern from nestin at 1d, doublecortin at 7–14d and MAP2 at 21d (Figure 1A). Caspase dependent and independent apoptosis was immunoassayed by active caspase-3 and annexin-V, respectively.

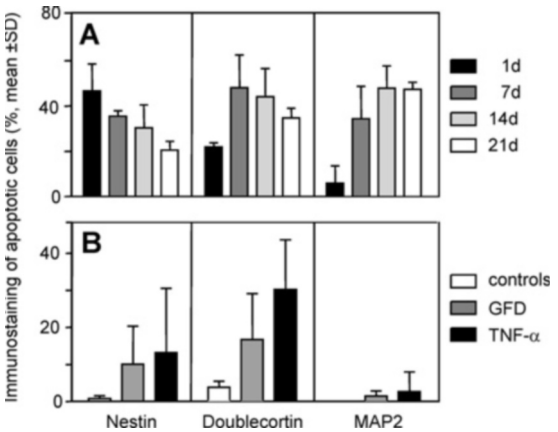

Figure 1.

Results: Caspase-3 dependent apoptosis was observed after GFD (27±7% of cells) and TNF-α (29±3%) but less after bacteria (5±3%) in mixed cell population at 7d. In contrast, apoptosis identified by annexin-V staining was induced by bacteria (25±6%) and to a lesser extent by GFD (16±7%) and TNF-α (17±3%). Vulnerability to apoptosis peaked at 7d when stem cells and immature neurons were specifically susceptible for caspase-3 dependent apoptosis induced by GFD 10±11% and 17±12% and by TNF-α 13±17% and 28±12% respectively (Figure 1B).
Conclusions: In a novel *in vitro* system of hippocampal neuronal differentiation, GFD and TNF-α induced caspase dependent apoptosis while bacteria induced caspase independent apoptosis. Stem cells and immature neurons are specifically vulnerable to undergo apoptosis in paradigms of BM. These results suggest that hippocampal injury in BM includes apoptosis of stem cells. The compromised regenerative potential of the hippocampus may contribute to the persistence of neurological deficits after BM.

O86 *Streptococcus suis*, an emerging zoonotic agent of meningitis, triggers different inflammatory signalling pathways by murine microglia

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Objective: *Streptococcus suis* type 2 is an important swine and human pathogen responsible for septicaemia and meningitis. We demonstrated that in the brain of infected mice with *S. suis,* microglia expressed increased levels of different pro-inflammatory genes. To expand our knowledge of the interactions between *S. suis* and microglia, we evaluated the signalling pathways underlying *S. suis*-induced pro-inflammatory events.
Methods: Mouse microglia line BV-2 was infected with a virulent wild type (WT) strain of *S. suis* or a panel of mutants altered at the capsule (CPS-mutant) or cell wall levels. Phagocytosis was quantified by an antibiotic protection assay. Cell supernatants were used to measure pro-inflammatory cytokines by ELISA and nitric oxide (NO) by the Griess reaction. Analysis from cell lysates, to evaluate inflammation-associated intracellular signalization pathways, was carried out by western blot using specific antibodies against iNOS, p-PKC, p-Tyr and mitogen-activated proteine kinase (MAPK) members p-JNK, p-ERK 1/2 and p-p38. Specific inhibitors of MAPK were used to confirm their participation in cytokine production. The involvement of the inflammatory transcription factor NF-kB was monitored by EMSA.
Results: Phagocytosis and cytokine studies showed that the CPS was the only relevant virulence factor modulating bacterial interactions with microglia. The CPS helped bacteria to resist phagocytosis and regulate the inflammatory response as it hides pro-inflammatory components from the bacterial cell wall. *S. suis* induced iNOS expression and further NO production from microglia. Cells infected with the CPS-mutant showed stronger phosphorylation profiles for PKC, Tyr and MAPK. Likewise, pharmacologic inhibition of MAPK abrogated TNF-α and MCP-1 production from infected cells. Finally, *S. suis*-induced NF-kB translocation was faster for cells stimulated with the CPS-mutant, suggesting that bacterial cell wall components are potent inducers of NF-kB.
Conclusions: Our data help to better understand the mechanisms underlying *S. suis* induction of inflammation in the brain, that in time would be useful to design more efficient anti-inflammatory strategies for meningitis.

Antimicrobial stewardship and antibiotic policies

O87 Antimicrobial stewardship programs in Spanish hospitals: a nationwide survey

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Objectives: 1) To describe the number, distribution and main features of antimicrobial stewardship programs (ASP) in Spanish hospitals. 2) To describe the Spanish Infectious Diseases (ID) community perceptions about antimicrobial (ABX) stewardship.
Methods: An online survey was designed. The link was distributed through the e-mailing lists of several working groups of the Spanish Clinical Microbiology and Infectious Diseases Society (SEIMC). The survey was anonymously submitted.
Results: Between Sept 15 2009 and Nov 15 2009, 110 surveys representing 76 hospitals were received from all over the country. 84 surveys were completely fulfilled (76%). Distribution of the replies per hospital size is described in table 1. Most of the respondents were either ID physicians (30%) or microbiologists (29%). 35/76 hospitals (41%) had an ongoing ASP. These programs were not homogenously distributed along the country but concentrated in 4 of the 17 Spanish Autonomous Communities, especially in Catalonia. 19/30 (63%) of the ASP were not limited to a specific clinical area of the hospital and could be considered "hospitalwide" programs. Most of these ASP (70%) have been working for more than 5 years. The most frequent principles of ABX stewardship implemented in these programs were: 1) ABX streamlining or de-escalation and 2) intravenous to P.O. switch, both present in 23/30 of the surveys followed by 3) monitoring of "strategic" ABX (22/30). 26/30 (86%) of respondents stated that any physician could initially prescribe all of the antimicrobials included in the formulary. The most frequently activity carried out in the setting of Spanish ASP was 1) ABX restriction 80% followed by 2) conferences (74%) and 3) therapeutic audit and feed-back TAFB (68%). Asked for the antimicrobials that should be closely monitored, carbapenems (26/39), linezolid (11/30) and fluoroquinolones (11/30). A majority of respondents (72%) was highly pleased with this task. The most efficacious activity in the setting were thought to be the TAFB (68% considered them highly efficacious) and automatic reminders of antimicrobial duration of therapy.
Conclusions: A minority (41%) of the surveyed hospitals in Spain has an ongoing ASP and large geographical variations were observed. Few of the ASP (30%) have been implemented in the last 5 years. TAFB was perceived as the most useful intervention in the setting of ASP. Carbapenems, linezolid and fluoriquinolones were considered the ABX most suitable to be monitored.

	⩾1000 beds	500–999 beds	<500 beds
Responses (%)	23 (20.9%)	35 (31.81%)	52 (47.27%)
Hospitals	11 (14.4%)	28 (36.8%)	37 (48.68%)

O88 Antimicrobial stewardship improves appropriateness of antimicrobial therapy prescription in a neurosurgical unit

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Objectives: To assess the impact of an Antimicrobial Stewardship Program (ASP) on antimicrobial usage and consumption, and patients' outcome in the neurosurgical setting of an Italian 850-bed tertiary hospital.
Methods: Between January 2007 and December 2008, a multidisciplinary Antimicrobial Management Team (AMT) provided a two-phase intervention in our 21-bed Division of Neurosurgery (NSU). In the observational period (2007), clinical, microbiological and pharmaceutical data of the year were collected, and reviewed with the ward medical staff thereafter. In the interventional period (2008), the AMT provided antimicrobial recommendation prescription on a regular and on-call basis, together with formulary restrictions and encouraged appropriate prophylaxis or targeted therapy whenever indicated. Recommendations were driven by updated specific pathogen resistance patterns and clinical pharmacology parameters; pharmaceutical data analysis was performed through the ESGAP ABC 3.1 program.
Results: The total number of admissions was 797 (bed-occupancy index = 0.87) in 2007 and 761 (bed-occupancy index = 0.82) in 2008, accounting for 6668.55 and 6302.52 bed-days, respectively. Among antimicrobial drugs commonly used for prophylaxis, the defined daily doses/100 bed-days (DDD) decreased from 558.33 to 406 for cefazolin, and from 363.66 to 239 for amoxicillin/clavulanate between 2007 and 2008. Among therapeutically used antimicrobials, vancomycin decreased from 205.5 to 37, meropenem from 360 to 249.5, levofloxacin from 804 to 564, and linezolid from 275.5 to 199, whereas ertapenem slightly increased (from 17 to 30) because of few invasive infections caused by ESBL-producing Enterobacteriaceae. However, mean length of stay decreased from 9.29 days to 8.90 between the two years, and no patients died in NSU due to infection-related causes during 2008. The grand total of antimicrobial expenditures also decreased from Euros 88,787.94 to 60,584.34.
Conclusion: The interventional policy by an AMT, based on the development of agreed upon prophylactic and therapeutic antimicrobial regimens, regular educational activities and microbiological and pharmaceutical monitoring significantly improved not only the appropriateness of antimicrobial prescription in this high-risk setting, but also the economical costs of antimicrobial acquisition.

O89 Efficacy and efficiency of a restrictive antibiotic policy on MRSA in the intensive care unit

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Objectives: To determine the efficacy and the efficiency of a restrictive antibiotic policy on the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the 18-bed general Intensive Care Unit (ICU) of an 850-bed tertiary care hospital in Italy.
Methods: Based on updated microbiological reports and clinical pharmacology parameters, the dedicated Antimicrobial Management Team pursued a more appropriate approach to antimicrobial prophylaxis and empirical therapy in ICU, either withdrawing ampicillin/sulbactam (AS) prophylaxis, or targeting vancomycin (VAN) therapy whenever indicated. Using an intervention time-series analysis, we first evaluated the efficacy of AS and VAN restrictions on their consumption, then combined this model with a transfer function model on use of other antibiotics and finally assessed the efficiency on the incidence of clinical isolates of MRSA from April 2004 to December 2007. The WHO ATC/DDD classification was used as reference normalized per 100 patient-days (PD).
Results: The intervention model demonstrated that the restrictive antibiotic policy yielded a statistical significant decrease of AS from 303 to 134 DDD/100 PD, and R2 was 64% in November 2003; six months later, VAN decreased from 36 to 11 DDD/100 PD (R2 of 46%). Five months after AS restriction, the MRSA incidence significantly decreased from 3.4 to 1.4 cases. The final model explained 42% of the incidence of MRSA over time, showing, conversely, that an increase of 1 DDD/100 PD of antibiotics increased the incidence of MRSA isolates from current level, i.e. 0.043 for ceftriaxone (significant impact at lag 2 and 3), and 0.012 for levofloxacin, at the same time.
Conclusion: This study shows that modelling antibiotic use can either drive a more appropriate empirical and targeted antimicrobial therapy, or inform policy makers about negative adverse effects of certain antibiotic

agents on selection of MRSA, and may ultimately control and prevent the misuse of antimicrobials. Restriction of several broad-spectrum antimicrobials might positively impact on MRSA, even if case-mix and the huge inhomogeneity of patients in general ICU may negatively affect the overall fitness of the model.

O90 Modelling the impact of antibiotic use on antibiotic-resistant *Escherichia coli* using population-based data from a large hospital and its surrounding community

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Objectives: We determined the temporal relationship between antibiotic use and the incidence of antibiotic-resistant *Escherichia coli* both in the inpatient and outpatient setting of the Swiss canton of Geneva.
Methods: An observational time-series analysis was performed to evaluate the incidence of non-duplicate clinical isolates of *E. coli* resistant to ciprofloxacin, trimethoprim/sulfamethoxazole and cefepime from January 2000 through December 2007. The resistance data were combined with a transfer function model of aggregated data on antibiotic use in both settings obtained from the hospital's pharmacy and outpatient billing offices.
Results: Ciprofloxacin resistance increased from 6% (2000) to 15.4% (2007; $P < 0.0001$) and cefepime resistance from 0.9% to 3.2% ($P = 0.01$). Trimethoprim/sulfamethoxazole resistance showed no trend (23.7%-25.8%). Total antibiotic use increased in both settings, while fluoroquinolone use increased significantly only among outpatients. A temporal effect between resistance in community isolates and outpatient use of ciprofloxacin (immediate and time lag, 1 month) and moxifloxacine (time lag 4 months) was observed, explaining 52% of the variance over time. The incidence of cefepime resistance in *E. coli*, as surrogate marker for ESBL, was correlated to ciprofloxacin use in the inpatient (lag 1 month) and outpatient (lag 4 months) settings and to the use of ceftriaxone (lag 0 months), piperacillin / tazobactam (lag 3 months) and cefepime (3 months) in the hospital (R2, 51%).
Conclusions: These results support efforts to reduce prescriptions of selected antimicrobial drug classes such as fluoroquinolones for reduction of resistant *E. coli* including ESBL and show the added value of time series analysis to better understand the interaction between community and hospital antibiotic prescribing.

Table. Multivariate transfer function model of fluoroquinolones use in the inpatient and outpatient setting and temporal relation with the incidence of non-duplicate clinical isolates of *E. coli*-resistance per 100 patient-days to ciprofloxacin. University of Geneva Hospitals, January 2000 to December 2007.

	CIP-R-CA, $R^2 = 0.52$				Cipro-R-HA, $R^2 = 0.18$			
Variable	Lag[a] (mo)	Parameter[b] (SE)	t-Statistic	P	Lag[a] (mo)	Parameter[b] (SE)	t-Statistic	P
Constant		−3.54 (0.24)	−14.76	0.0000		−3.34 (0.01)	−42.86	0.0000
Ciprofloxacin, outpatient use	0	1.31 (0.49)	2.68	0.0089				
	1	1.01 (0.49)	2.08	0.0406	1	0.82 (0.30)	2.76	0.0069
Moxifloxacin, outpatient use	4	0.44 (0.16)	2.72	0.0081				
Autoregressive term[c]	1	0.31 (0.11)	2.89	0.0050	1	0.24 (0.10)	2.43	0.0170
Moving average term[d]	8	0.36 (0.11)	3.24	0.0017				

CIP-R-CA: Community acquired ciprofloxacin resistant *E. coli* isolates, CIP-R-HA: hospital acquired ciprofloxacin resistant *E. coli* isolates.
[a]Delay (months) necessary to observe the effect. [b]Size and direction of the effect. [c]The autoregressive term represents the past value of the resistance. [d]The moving average term represents disturbances or abrupt changes of resistance.

O91 Reduction in ciprofloxacin use in a university hospital correlates with increased susceptibility to both quinolone and β-lactam antibiotics in Gram-negative organisms

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Objectives: An antibiotic policy revision was implemented at our institution, a university hospital in Belfast, in July 2008. Its purpose was to substitute ceftriaxone and ciprofloxacin, with B-lactam antibiotics. The aim of this study was to investigate whether, following the policy change, there was a:

- change in usage of piperacillin–tazobactam (pip–tazo), aztreonam, ceftriaxone, ciprofloxacin and meropenem, based on defined daily doses (DDD) prescribed;
- shift in the proportion of Gram-negative organisms susceptible to these antibiotics;
- significant relationship between susceptibility and usage of these antibiotics.

Methods: The first isolate of all Gram-negative organisms recovered from blood cultures, sputum and urine of hospitalised adults, between January 2008 and August 2009, were included. The proportion of isolates reported susceptible, before and after the policy change, were compared by χ^2 test; DDD before and after were compared using independent t-test. The association between DDD prescription and susceptibility was tested using Pearson correlation and linear regression.
Results: Ciprofloxacin use decreased ($p < 0.001$) and aztreonam use increased ($p = 0.03$) following policy revision. There was no change in meropenem, ceftriaxone or pip–tazo use. In all, 5445 isolates were evaluated; the proportion susceptible to ciprofloxacin ($p < 0.001$), aztreonam ($p < 0.001$), and pip–tazo ($p = 0.049$) increased. A significant inverse linear association between ciprofloxacin use and susceptibility was detected ($p < 0.001$; correlation $p < 0.001$). Furthermore, pip–tazo, aztreonam and meropenem susceptibility were inversely correlated with ciprofloxacin use (univariate pip–tazo $r = -0.39$ $p = 0.045$; aztreonam $r = -0.69$ $p < 0.001$; meropenem $r = -0.53$ $p = 0.008$), with a linear relationship apparent (pip–tazo $p = 0.089$; aztreonam $p = 0.001$; meropenem $p = 0.016$). Surprisingly, there was a significant positive linear association between aztreonam use and susceptibility ($r = 0.61$ $p = 0.004$).
Conclusions: These data suggest that an antibiotic policy revision, successfully reducing ciprofloxacin use, correlated with an increase in ciprofloxacin susceptibility among Gram-negative organisms. Furthermore, this was also associated with an increase in susceptibility to B-lactam antibiotics, in spite of increased use of aztreonam. Therefore, reducing quinolone exposure within a population appears to exert a favourable effect on both quinolone and B-lactam susceptibility of Gram-negative organisms.

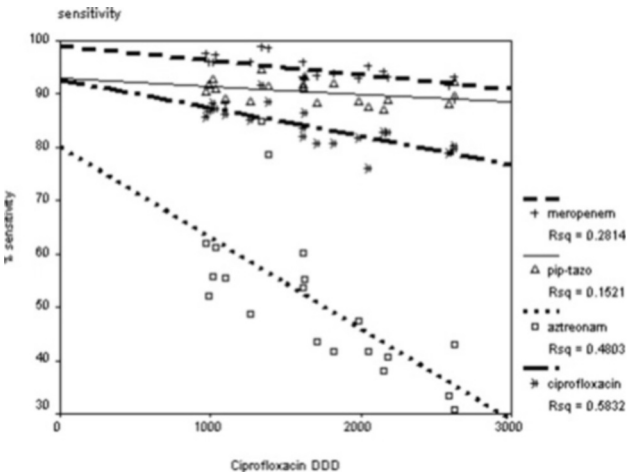

Figure. Correlation of ciprofloxacin DDD with ciprofloxacin and β-lactam.

O92 Life without ciprofloxacin: implementation and consequences of a fluoroquinolone ban for a tertiary referral hospital

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Objectives: An outbreak of *Clostridium difficile* infection (CDI) due to PCR ribotype 027 affecting 42 patients occurred in our hospital between July 2008 and March 2009. The reported association between

fluoroquinolone use and *C. difficile* colitis and evidence that *C. difficile* 027 isolates tend to be fluoroquinolone-resistant, prompted the outbreak control team to recommend a complete ban on the prescription of fluoroquinolone antimicrobials as one component of the multifactorial outbreak management programme, effective September 2008. This report describes the practicalities of introducing a fluoroquinolone ban, the efforts to maintain the ban and some of the observed consequences of the ban.

Methods: The hospital's clinical microbiology team, surveillance scientist and antimicrobial pharmacist collected data regarding the impact of this ban on rates of inpatient *C. difficile* infection, fluoroquinolone resistance in *E. coli* bacteraemia isolates and the use of other antimicrobial agents.

Results: At the peak of the outbreak of CDI, there were on average 16 new cases diagnosed per month. Following closure of the outbreak, the number of new cases of CDI has declined to an average of 7.8 per month. Compared with 2007, fluoroquinolone use for inpatients during 2008 declined by 59% (19.07 DDD/100BD to 7.82 DDD/100BD). However, for the same period, meropenem use increased by 43% (1.34 DDD/100BD to 1.92 DDD/100BD). The proportion of fluoroquinolone resistance in *E. coli* isolates from bloodstream infections in our hospital has decreased from 25% in 2008 to 5% for quarters 1 & 2 2009.

Conclusions: Implementation of a fluoroquinolone ban has proved challenging, but has rewarded with a reduction in cases of CDI and fluoroquinolone resistance in *E. coli.* Such an intervention may be a useful adjunctive measure in the management of outbreaks of infection due to *Clostridium difficile* ribotype 027. Of concern, is the increased use of broad spectrum agents such as meropenem. Further effort is required to limit the use of such agents, particularly in the context of the recent emergence of carbapenemase producing Enterobacteriaceae and the lack of new anti-Gram-negative agents.

O93 An audit of fluoroquinolone prescription in a paediatric hospital

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Objectives: To evaluate the prescription of fluoroquinolones (FQ) for pediatric infections.

Methods: An audit of FQ prescription was conducted between 1st December 2007 and 30th June 2008 in Necker-Enfants Malades University hospital in Paris. Children admitted and receiving systemic FQ were included in the study. Descriptive data concerning patients' characteristics (immune status, cystic fibrosis), the present FQ regimen (drug, indication and bacteriologic findings, justification for use, concomitant drugs) were evaluated by 3 experts of infectious disease and 1 pharmacist. Statistical analysis was performed using Epi info version 6, CDC Atlanta.

Results: 52 oral (53.1%) and 46 (46.9%) intravenous FQ prescriptions were collected. Prescriptions mainly originated from general pediatric wards (25.4%), immuno-hematology department (20.4%), intensive care units (12.2%) and cardiac surgery unit (11.2%).

Among these 98 children, median age was 10 years. 45 (45.9%) had an underlying condition, CF (21.4%) and immune deficiency such as neutropenia (12.2%), use of any immunosuppressive agent (12.2%). The indications for FQ prescription to patients with CF (21) were bronchopulmonary *P. aeruginosa* infection or prophylaxis (20/21). Among 77 patients without CF, of which only 14 had *P. aeruginosa* infection, the main infection sites were bronchopulmonary (25), post-operative (10), urinary tract (8), gastrointestinal tract (6), septicemia (6), bone or joint (4) and cerebral (4), endocarditis (2). 61 (62.2%) infections were nosocomially aquired. 22 of the 35 community acquired were health care associated. Bacteriological confirmation was isolated in 60 (61.2%) patients.

52 prescriptions were considered as adequate FQ use, of which 36 were mandatory, 16 as adequate indication based on clinical and/or microbiologic findings. 35 of 46 inadequate prescriptions might have had an alternative antibiotic to FQ, 11 were unnecessary. Median ciprofloxacine dosage was 20 mg/kg/24 h. 46 patients received an excessive dose, while 7 had insufficient treatment. 87.8% patients had a concomitant drug use.

Appropriate prescription of FQ was associated with *P. aeruginosa* infection in CF patients ($p = 0.0001$, OR = 12.67, 95% CI 2.52–86).

Conclusion: More than a half FQ prescription in pediatric patients was adequate and mandatory. The problems of inadequate prescriptions focus on the dosage and that FQ could be replaced by an alternative antibiotic. Appropriated prescriptions were associated with *P. aeruginosa* infection in CF patients.

O94 Do published studies provide accurate estimates of the association between antibiotic exposure and acquisition of antibiotic-resistant bacteria?

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Objectives: Antibiotic stewardship is usually included in a multifaceted approach to combat the spreading of antibiotic resistant bacteria (ARB) into the healthcare setting. Numerous papers have demonstrated that prior antimicrobial drug exposure is a strong risk factor for infection due to ARB. However, the association between antibiotic therapy and the acquisition of ARB is still unclear and it is often confounded by scarce data on antibiotic usage. Our objectives were to define major limits of the available evidence and to explore the sources of heterogeneity.

Methods: Two meta-analyses were performed to determine whether antibiotic exposure is a risk factor for the isolation of ARB. Target bacteria were: methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenems-resistant *Acinetobacter baumannii* (CRAB). The I2 test was calculated to assess whether results varied no more than might have been expected by the play of chance. The subgroup analysis was performed by stratifying sampling frame for inclusion, definition of case subjects and controls, study design, geographic area of the study, presence of adjustment of covariates and length of time in which antibiotic exposure was detected. A significant heterogeneity was considered for $I2 > 50\%$.

Results: Seventy-eight studies (60 related to MRSA and 18 studies to CRAB) were considered eligible for inclusion. All studies were cohort, case–control or prevalence survey. Patients who had taken antibiotics had a risk increased by 1.8 (95% CI, 1.7–1.9) and by 1.3 (95% CI, 1.2–1.4) fold of acquiring MRSA and CRAB, respectively. Significantly heterogeneity was detected for study design, definition of controls, sampling frame for inclusion, endemic / epidemic setting, adjustment for covariates. A regression analysis revealed that the heterogeneity was linked to the length of time in which antibiotic exposure was detected before isolation, selection of the control group, and sampling frame for inclusion.

Conclusions: Our analysis confirms that the quality of the evidence available on the association between antibiotic therapy and the acquisition of antibiotic-resistant bacteria is poor. Specific guidelines focused on methodological issues to address the epidemiology of antibiotic-resistant infections are urgently needed.

O95 Divergent intentions to use a local antibiotic guideline: a theory of planned behaviour survey

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Background: In order to improve antimicrobial practice, correctly identifying antibiotic guideline barriers, and assessing their relative importance are important factors. The Theory of Planned Behaviour (TPB) permits such assessment and has been used already for evaluating antibiotic use (1). According this theory, the intention driving guideline use, is fuelled by three factors: attitude; subjective norm (SN) (perceived social pressure regarding guidelines); and perceived behavioural control (PBC) over the guideline. Using a TBP-based questionnaire, we wanted to study how guideline use is affected in our hospital.

Methods: Based on earlier observations, a questionnaire was constructed, modified to account for habit strength (2). After pilot testing, the survey was distributed among physicians in a major teaching hospital.
Results: Out of 393 contacted physicians, 195 completed questionnaires were received (50.5% corrected response rate). Using multivariate analysis, habit strength ($\beta = 0.186$; $P = 0.022$) and PBC ($\beta = 0.171$; $P = 0.036$), were significant determinants for overall intention. A moderator effect of respondents' position (staff member vs. resident) was found with staff members' intention influenced by habit strength ($\beta = 0.281$; $P = 0.038$), and residents' intention by PBC ($\beta = 0.220$; $P = 0.040$) only. Other factors were not significant. Looking for specific issues, education on antibiotics and guidelines was rated unsatisfactory.
Conclusions: These divergent origins stipulate different approaches for improvement. With staff members experiencing a high influence of previous routine and habits, preferred interventions for this group are education, focused on changes in prior antibiotic practice and their causes, and reminders. Residents' intention is guided mainly by external influences and the amount of self-confidence, making feedback, convenient guideline formats and thorough guideline familiarization more suitable.

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O96 Temporal effects of a restrictive antibiotic policy on hospital-acquired *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* and extended-spectrum β-lactamase producing coliforms in a district general hospital

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Objectives: Following a persistent problem with cases of *Clostridium difficile*, a restrictive antibiotic policy was imposed on a 400-bedded district general hospital. This policy banned the use of cephalosporin and quinolone antibiotics unless released by the microbiologist. Hospital pharmacists upheld the policy on the wards and stocks of restricted agents were removed from all wards except intensive and emergency care.
Methods: The policy was introduced following a brief educational campaign aimed at all doctors. From six months before, until 14 months after the policy began, we calculated the monthly consumption of all antibiotic classes in defined daily doses (DDDs) per 1000 patient-occupied bed-days (1000 pt bds) for the whole hospital. In addition, we determined each new case of hospital-acquired *C. difficile*, meticillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase-producing coliform (ESBLs) infection for the whole 22-month period. These were calculated as the number of cases per month per 1000 pt bds.
Results: During the six months before the policy was introduced, the hospital experienced an average monthly rate of 1.009 MRSA cases/1000 pt bds; 2.398 *C. difficile* cases/1000 pt bds; and 1.480 cases ESBLs/1000 pt bds. The average monthly consumption of ceftriaxone over the same period was 46.213 DDDs/1000 pt bds and for ciprofloxacin was 109.804 DDDs/1000 pt bds. During the six month period at the end of the study, ten months after the policy was introduced, infection control identified a monthly average of 0.938 MRSA cases/1000 pt bds; 1.424 *C. difficile* cases/1000 pt bds; and 1.134 cases ESBLs/1000 pt bds. The average monthly consumption of ceftriaxone over the final six months had dropped by 95% to 2.113 DDDs/1000 pt bds and for ciprofloxacin by 76% to 26.672 DDDs/1000 pt bds. There were corresponding increases in consumption of amoxicillin and gentamicin, no change in consumption of piperacillin–tazobactam, and a small decrease in carbapenem antibiotics. We have submitted the data for time-lag modelling.
Conclusion: A restrictive antibiotic policy had a profound effect on the number of cases of hospital-acquired *C. difficile* (reduction of 40%), less effect on ESBLs (reduction of 23%) and little effect on MRSA (7% reduction). Whilst it is important to reduce antibiotic consumption as far as possible, it is clear that additional infection control interventions are required for hospital-acquired infection, particularly MRSA.

Molecular typing and epidemiology

O97 Microarray analysis and antibiotic susceptibility patterns among 131 *Staphylococcus aureus* causing infective endocarditis in France in 2008

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Background: The changing epidemiology of staphylococci worldwide, with the emergence of community-acquired MRSA called for a thorough analysis of the *S. aureus* isolated during the French national survey on infective endocarditis (IE) conducted in 2008.
Methods: We analyzed 131 non-duplicate staphylococcal isolates collected during the 2008 IE French survey. Genomic identification, antibiotic susceptibility testing and search for mecA gene were performed. MRSA strains were subjected to population analysis for determination of glycopeptide heteroresistance. All *S. aureus* were spa-typed and 114 MSSA were analyzed by a DNA microarray covering 185 distinct genes (300 alleles), including genes encoding virulence factors and resistance genes.
Results: *MRSA*: 17 MRSA (13%) belonging to only 5 spa-types and 3 sequence-types (ST) were detected demonstrating a high clonality. They were frequently resistant to levofloxacin (88%), kanamycin and tobramycin (47%), and to erythromycin (35%). Four of the MRSA were categorized as hVISA by population analysis. No strain belongs to any CA-MRSA clone.
MSSA: The 114 MSSA isolates were seldom resistant to erythromycin (16/114, 1.4%) and almost fully susceptible to all other antibiotics. Phenotypic results were highly correlated with genomic data from microarray. All MSSA isolates were assigned to 19 ST. Their ST diversity evaluated by the lamba modified Simpson was not different when compared to a set of 114 *S. aureus* invasive isolates collected throughout France during the EARSS study in 2006–2007 (88.31% vs 91.09%). Surprisingly we noticed that 8 isolates belonged to ST398. Genomic DNA microarray comparison of the 114 MSSA IE isolates with 152 MSSA nasal carriage isolates highlighted that several specific determinants or gene alleles (e.g., bone sialoprotein, elastin binding protein, modulins) were preferentially harbored by MSSA IE isolates.
Conclusions: The genetic diversity of *S. aureus* strains from IE suggests that there is no specific background strain associated with IE; however some virulence traits are highlighted; it remains to be determined whether some specific genetic traits of the human host favor the occurrence of *S. aureus* IE. MRSA, including CA-MRSA are infrequent.

O98 Improved discrmination of highly-clonal ST22-methicillin-resistant *Staphylococcus aureus* (MRSA)-IV isolates achieved by combining spa, dru and pulsed field gel electrophoresis typing data

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Objective: The pandemic ST22-methicillin resistant *Staphylococcus aureus* (MRSA)-IV strain has been endemic in Irish hospitals since 2002, where it is designated as antibiogram-resistogram type-pulsed-field group (AR-PFG) 06–01 and is similar to the UK epidemic strain EMRSA-15. Differentiating isolates of this strain is difficult as they exhibit a limited number of pulsed-field gel electrophoresis (PFGE)

patterns and spa types. This study investigated whether combining PFGE and spa typing with DNA sequencing of the SCCmec-associated direct repeat unit (dru typing) would significantly improve isolate discrimination.
Methods: One hundred and seventy-three MRSA isolates recovered in one Irish hospital during 2007 and 2008 were investigated using AR, PFGE, spa, dru and SCCmec typing. One isolate representative of each of the spa types identified underwent multilocus sequence typing.
Results: Ninety-seven percent of isolates (168/173) exhibited AR-PFG 06–01 or closely related AR patterns and 163 of these harboured SCCmec IVh. Isolates representative of the 17 PFG-01 spa types were identified as ST22. Combining PFGE, spa and dru typing data significantly improved discrimination of the 168 PFG-01 isolates yielding 65 type combinations with a Simpson's Index of Diversity (SID) of 96.53 compared to a) pairwise combinations which yielded 37, 44 and 43 type combinations with SIDs of 90.84 for spa and dru, 91.00 for spa and PFGE and 93.57 for dru and PFGE; respectively, or b) individual typing methods which yielded 21, 17 and 17 types with SIDs of 66.9, 77.8 and 81.34 for spa, dru and PFGE, respectively. Analysis of epidemiological information for a subset of PFG-01 isolates validated the relationships inferred using combined PFGE, spa and dru typing data.
Conclusions: This study demonstrates that the combination of PFGE, spa and dru typing improves discrimination and epidemiological tracking of highly clonal ST22-MRSA-IV isolates.

O99 External quality assessment of genotyping techniques for methicillin-resistant *Staphylococcus aureus*

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Background: After detection of meticillin resistant *Staphylococcus aureus* (MRSA), genotyping may be necessary to proceed with infection-preventive measures. This external quality assessment study determines the performance of genotyping techniques for MRSA in participating laboratories and was organised by Quality Control for Molecular Diagnostics (QCMD) (www.qcmd.org).
Methods: The EQA panel for MRSA genotyping was distributed to 19 participants in 8 countries in August 2009 and consisted of 10 samples (2 identical, 3 genetically related and 5 unique, as determined originally with pulsed-field gel electrophoresis [PFGE]) of viable MRSA strains in Müller Hinton broth. A different letter signified the detection of a different genotype, whereas a different number signified the detection of a different subtype. All data were reported in relation to the reference strain in panel sample MRSATP09–01.
Results: Out of the 19 potential participants, 14 (74%) responded. Four of the non-responders withdrew officially indicating 'panel used for research' (n = 1) and 'assay not offered' (n = 3). The majority of datasets were generated by PFGE (n = 11), with the remainder generated by AFLP (n = 2) and spa typing (n = 2). Seventy-three percent of participants typed all samples correctly, all with PFGE. Results obtained from spa-typing and amplified fragment length polymorphism (AFLP) were found to be less discriminative than those obtained with PFGE.
Discussion: We present the first EQA programme for the genotyping of MRSA. PFGE was implemented by most of the participating laboratories. However, most protocols proved to be suboptimal, resulting in inferior resolution in the higher or lower fragment regions. This suggests that further assay optimisation is required. The lack of resolution was most evident with the closely related MRSA strains in the panel. Results generated using AFLP and spa-typing showed less discriminatory power compared to PFGE. Participants reported a range of criteria for determining genotype and subtype. The guidance according to Tenover et al was the most prominent method. Future EQA distributions will gather information on the cutoff values used by participants. To improve the performance and quality of MRSA genotyping and subtyping, both laboratories and manufacturers should be encouraged to participate in EQAs. The availability of EQA panels for detection and typing should also be developed for other important (nosocomial) infectious agents.

O100 Usefulness of DiversiLab rep-PCR system for typing and follow-up of *Pseudomonas aeruginosa* strains colonizing cystic fibrosis patients: comparison with PFGE and MLST techniques

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Objective: rep-PCR typing method has been recently proposed as alternative to the classical PFGE for Clinical Microbiology Laboratories. This method demonstrated their utility in outbreaks typing whereas non-experience in the follow-up of chronically colonized patients is available. The aim of this work was to evaluate the ability of the rep-PCR DiversiLab system (bioMérieux) to discriminate a well-typed collection of clinical *P. aeruginosa* strains from cystic fibrosis (CF) patients.
Material and Methods: A collection of 49 *P. aeruginosa* strains recovered from sputum samples of 24 CF-patients (1994–2009) were studied. Strains were classified as first colonizer (n = 24), sporadic or intermittent (n = 13), and persistent colonizers (n = 12). First colonizer was defined as the first *P. aeruginosa* isolate detected from sputum in each CF-patient. In four patients, first colonizers were identical to the follow-up isolates, so were also classified as persistent. Genetic relationships were analyzed by PFGE-SpeI and MLST and after compared with those obtained by DiversiLab, which generates patterns by use of microfluidic chips. Follow-up of CF-patients include comparison of the first colonizer, sporadic and chronic isolates as well as possible persistence and cross-colonization.
Results: Among the 24 first colonizer strains, 22 PFGE patterns and 21 unrelated STs were detected. rep-PCR DiversiLab confirmed these data with discrepancies only in one strain. Two strains belonging to ST312 were classified by DiversiLab as unrelated, and another two strains grouped in ST274 had different PFGE and DiversiLab patterns. The 13 sporadic strains were obtained from 7 CF-patients. Genetic relationship was not detectable among them using either PFGE or MLST tools, and DiversiLab confirmed this pattern of heterogeneity except in one strain. Chronic colonizers (12 + 4 first colonizer strains) were obtained from 4 CF-patients (2–5 strains per patient). The three typing methods agreed with the result that particular host-specific stable clones were detected in different patients, with no genetic relation between them.
Conclusions: DiversiLab system based on rep-PCR technique is a reliable method useful for Clinical Microbiological Laboratories in the follow-up and colonization dynamic of CF-*P. aeruginosa* strains. This technique is considerably less laborious than the classical PFGE and MLST and genetic results for our first colonizers, sporadic or persistent strains are comparable.

O101 Sequence types of carbapenem-resistant *Acinetobacter baumannii* strains in Greece during the last decade

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Objectives: During the last decade, carbapenem-resistant *Acinetobacter baumannii* isolates are increasingly isolated; in the ICUs of many regions worldwide such isolates currently represent the vast majority of *A. baumannii* recovered from hospital infections. The aim of the present study was to analyse the genetic relatedness of multidrug resistant *A. baumannii* isolates recovered from ICU patients in different regions in Greece.
Methods: One hundred *A. baumannii* isolates that were derived from 2001 to 2009 from 10 hospitals located in four different geographical regions in Greece were included in the study. Antimicrobial susceptibilities to carbapenems were determined by the reference agar dilution method. The isolates were tested by PFGE and sequence-based typing (ST) using ompA, csuE and blaOXA-51-like sequences.
Results: Eighty-one (81%) of the isolates were carbapenem-resistant. Eleven different ST-types were revealed. Thirty-four isolates (34%) belonged to ST-group 2 (corresponding to EU clone I), 55 (55%) to ST-group 1 (EU clone II) and the remaining 11 were sporadic isolates

belonging to 10 unique ST-types. The latter ST-types were not assigned to ST-groups, as not yet being epidemic. This is in accordance with previous reports showing that the majority of *A. baumannii* strains circulating in Europe belonged to ST-groups 1 and 2. Within the same institution, isolates with the same PFGE-profile belonged to the same ST-type, although some isolates of the same ST-type exhibited different PFGE profiles and isolates of different ST-types tended to have unrelated PFGE profiles. Sporadic isolates that had unique ST-types had also unique PFGE profiles. ST-groups 1 and 2 included isolates that were derived from all geographical regions and comprised both carbapenem-resistant and susceptible isolates. The majority of isolates recovered prior to 2003 belonged to ST-group 2, while those isolated after 2004 more commonly belonged to ST-group 1.

Conclusions: The epidemic of multidrug and carbapenem-resistant *A. baumannii* in Greece was sustained by the spread of distinct genotypes belonging mainly to ST groups 1 and 2, which also included carbapenem-susceptible isolates and in a lesser extent to other sporadic ST-types.

O102 Differentiation of Austrian *Salmonella* serotypes using high-resolution melting curve analysis

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Objectives: Effective epidemiological surveillance requires the accurate subtyping of strains. The standard method for differentiation of *Salmonella* strains is serotyping. Despite the utility of serotyping, testing with a complete set of sera is time consuming and requires a well trained technician; problems associated with antiserum production and occurrence of strains for which a serotype antigen cannot be detected prompted reference laboratories to pursue molecular approaches for serotyping.

The aim of this study was to assess the usefulness of high-resolution melting (HRM) curve analysis on the LightCycler 480 PCR system as a tool for accurate and fast molecular typing of *Salmonella enterica* subsp. *enterica* strains.

Methods: A collection of *Salmonella* isolates representing diverse serotypes was recently analysed using Amplified Fragment Length Polymorphism (AFLP) (1). A phylogenetic cluster comprising the serotypes Blockley, Virchow, Braenderup, Manhattan and Muenchen was chosen to evaluate the potential of HRM analysis for molecular typing. A 171 bp fragment of the gyrase B gene (gyrB) was amplified for HRM curve analysis on a LightCycler 480 instrument (Roche Diagnostics, Penzberg, Germany).

Results: HRM curve analysis of a 171 bp amplicon of the gyrB gene resulted in five different melting curves (Figure 1), and thus allowed the rapid and accurate discrimination of the investigated serotypes.

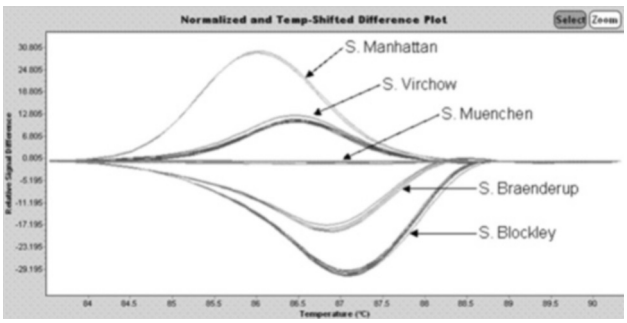

Figure 1. Melting curve profiles of the tested *Salmonella* serotypes.

Conclusion: HRM analysis is a new single-step closed-tube screening method for fast mutation detection and can therefore be used to discriminate even genetically closely related samples. HRM analysis has the potential to complement the classical serotyping of *Salmonella* isolates due to its discriminatory power and simplicity.

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O103 Direct high-resolution genotyping of *Chlamydia trachomatis* positive swabs from women in Southampton using ompA and three variable-number tandem repeats

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Objectives: Genital chlamydial infection is the commonest diagnosed sexually transmitted infection in the UK. *Chlamydia trachomatis* infections are caused by strains which fall into two pathovars: 'serovars' of lymphogranuloma venereum (LGV) and the genitourinary 'serovars' D-K. Although these serovars can be discriminated by outer membrane protein gene (ompA) sequencing (genotyping) or multi-locus sequence typing (MLST), neither affords the high-resolution genetic typing required for local epidemiology and accurate contact-tracing. Therefore our objectives were to develop and apply a new high resolution genetic typing technique to studying the molecular epidemiology of *C. trachomatis* in positive swabs from our local genitourinary medicine (GUM) clinic.

Methods: 162 endocervical swabs were taken at the Southampton GUM clinic and tested by routine diagnostic PCR for the presence of *C. trachomatis.* Positive samples were genotyped by use of a variable number tandem repeat (VNTR)-ompA sequencing technique. Isolates were cultured from the positives where possible.

Results: Of the 162 samples, 86 were fully typed by VNTR-ompA. Only one mixed infection (E & F) in one sample was confirmed. The commonest genotypes were D, E & F, comprising 20%, 45% and 15% of the genotyped positives respectively. Within each of these genovars there were multiple VNTR sub-types. 69 of the swabs yielded culturable isolates able to passage multiple times.

Conclusions: This is the first comprehensive fine molecular epidemiology genetic typing survey of *C. trachomatis* in the UK. Amongst the common genotypes, there are a significant number of defined sub-types, which may reflect backgrounds of particular demographics relating to age group, geography, high-risk sexual behaviour, and sexual networks.

O104 Comparison of *Listeria monocytogenes* strains from food and human origin by amplified fragment length polymorphism

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Objectives: *Listeria monocytogenes* is a foodborne pathogen, responsible for neurological, systemic and gastro-intestinal disease with a mortality up to 30%. For the investigation of *L. monocytogenes* epidemiology highly discriminatory typing methods are needed. The aim of our study was to develop an Amplified Fragment Length Polymorphism (AFLP) for *L. monocytogenes,* analyze isolates from clinical human and food origin and determine the possibility of differentiation between human and food isolates by AFLP.

Methods: Based on genome sequence analysis we selected restriction enzyme combination HindIII-G and HPyCHIV4-C. With this combination, a collection of 279 *L. monocytogenes* strains was typed, consisting of 168 human clinical isolates from the Netherlands Reference Laboratory for Bacterial Meningitis (RBM), 111 food isolates from the Food and Consumer Product Safety Authority (VWA), 3 complete genome sequenced strains (ATCC BAA-679 (=EGDe), F2365, HCC23) and *L. monocytogenes* type strain ATCC 15313. As such, this *L. monocytogenes* collection is one of the largest collections in the world comparing both clinical and food isolates.

Results: AFLP patterns of the 279 *L. monocytogenes* isolates were grouped in two major clusters and a few individual isolates. Cluster I included 100 human isolates and 37 isolates of food origin. The human

cluster I isolates mainly consisted of the virulent serotype 4b (63/100, 63%) and serotype 1/2b (14/100, 14%). The cluster I isolates originating from food consisted mainly of serotype 1/2b (15/37, 40.5%). These food isolates formed a subcluster within cluster I. Cluster II included 68 human and 74 food isolates, mainly serotype 1/2a (69.1% and 35.1%, respectively).
Conclusion: AFLP allows discrimination of *L. monocytogenes* in two major clusters. The two clusters show segregation of the virulent 4b serotype into AFLP cluster I, whereas serotype 1/2a predominates in AFLP cluster II. Within cluster I, it is possible to differentiate most food 1/2b isolates from human isolates.

O105 Investigation of an outbreak of CTX-M-15-producing *Escherichia coli* of sequence types 131 and 1441 in a neonatal surgical ward: comparison of typing methods

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Objectives: The spread of *E. coli* producing the CTX-M-15-type of extended-spectrum β-lactamase (ESBL) was ongoing in a surgical ward caring for newborns since, at least, September 2008 and was finally recognised in late December. Various typing methods were applied and compared with pulsed-field gel electrophoresis (PFGE) to verify the outbreak and to determine the number of affected children.
Methods: Subsequent to clinical sampling, 125 children hospitalised September-December were screened for ESBL-bacteria in stool. January-June 2009, newly admitted children were screened at admission and twice weekly. 51 ESBL-*E. coli* isolates were detected in 26 children, of which 6 later were found to carry isolates of several PFGE-types. The 51 isolates were typed by PFGE, multiple-locus-variable number tandem repeat analysis (MLVA), a "mini" multiple-locus-sequence typing (mMLST) method (fumC, purA and dnaJ genes) and by Phene Plate (PhP) biochemical fingerprinting.
Results: When the outbreak was revealed, five children had developed infections with ESBL-*E. coli* of two PFGE-types, A (ST 131) and B (ST 1441), later determined to be the outbreak strains. One or both types spread to a total of 21 children. Altogether, 38 isolates (20 children) were of type A and 7 isolates (5 children) of type B. In addition six children carried isolates of six distinct PFGE-types (C-H), only found in one child each. MLVA generated the same strain differentiation profile as PFGE. mMLST accurately detected the same STs of PFGE-types as detected by standard MLST (http://mlst.ucc.ie/mlst/dbs/Ecoli), although it did not differentiate ST 131 isolates of different PFGE-types (A and C). PhP-typing differentiated isolates such that little correlation was observed with PFGE. By monitoring resistance patterns of isolates we could not predict the identity of isolates.
Conclusion: If transmission is ongoing for an extended time period, several types of ESBL-producing bacteria may be detected in an outbreak and all isolates, including screened as well as repeat isolates, should be typed to identify affected patients. Only genetic methods gave satisfactory typing results in investigating this outbreak. MLVA generated identical results as those of PFGE and is, thus, attractive, being faster, less-costly and producing results that are easier to communicate. The mMLST, although accurately detecting the STs, despite using only three house-keeping genes, was less discriminatory than PFGE or MLVA.

O106 Deep sequencing for accurate and high-throughput HPV genotyping in clinical samples

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Objectives: Human papillomavirus (HPV) typing is useful for studies on HPV transmission, natural history, pathogenesis, and prevention and for clinical management of HPV-related lesions. HPV typing methods based on Sanger sequencing or reverse hybridization show poor accuracy in multiple infections. Moreover, discordant results have been reported when using different typing methods even in single infection. Aim of the study was to develop an HPV typing method based on 454 Life-Sciences ultradeep pyrosequencing in order to dissect the contribution of each HPV type in multiple infections and to identify possible HPV sequence variants.
Methods: A group of 50 HPV-DNA positive cervical samples, including 40 with multiple infections, were selected for deep pyrosequencing. For each cervical sample, a library of amplicons targeting a conserved region of HPV-L1 was generated and sequenced in multiplex reactions on a 454 Life-Sciences platform. Multiple alignment of sequences was performed with Clustalw2, followed by clustering analysis based on sequence identity (Jalview 2.4). A representative sequence was selected for each cluster and aligned with the non redundant database of nucleotide sequences, in order to identify either multiple infections or contaminant genomic DNA. Results from deep sequencing were compared with Sanger sequencing and with the Inno-LiPA HPV genotyping test.
Results: A mean coverage of 1228 (range, 234–3202) sequences was obtained for each sample analyzed by deep sequencing. In 3 cases classified as single infection by both Sanger sequencing and Inno-LiPA, deep sequencing revealed the presence of multiple HPV types, while in 12 cases classified as multiple infection, Inno-LiPA and deep sequencing gave discordant typing results. An overall analysis of results showed the sensitivity of HPV deep sequencing was higher than other typing methods, since it could detect HPV types representing less than 1% of sequences in multiple infections, at variance with Sanger sequencing, which failed in most cases of multiple infection, and the Inno-LiPA assay, which could not detect HPV types representing less than 8% of sequences in multiple infections nor those not included in the probe set. Moreover, deep sequencing allowed identification of unclassified HPV types and detection of HPV sequence variants within types.
Conclusions: Deep sequencing may be a sensitive and accurate high-throughput method for HPV genotyping in clinical samples.

Antifungal therapy under the microscope: from fungus to mouse to man (Symposium supported by Gilead)

S108 Do animal models help predict host responses to fungal infections and antifungal treatments? Choosing therapy on the basis of efficacy not safety

J. Adler-Moore (Pomona, US)*

Clinical outcomes from invasive fungal infections involve a complex interplay between the infecting organism, antifungal treatment and the host immune response. Factors such as the host's neutropenic status are well known to influence outcome and may be of particular relevance if the antifungal treatment is fungistatic rather than fungicidal, but the immune response involves far more than neutrophils alone. Cytokine changes may correlate with both clinical response and clinical failure. In this symposium the faculty will show how changes *in vitro* may correlate with responses *in vivo* in animal models and therefore predict the response in the human host.

MRSA: from molecular epidemiology to molecular screening (Symposium supported by Roche Molecular Diagnostics)

S111 Differentiation between human epidemic and animal associated MRSA strains by LightCycler melting curve analysis

U. Reischl (Regensburg, DE)*

Objectives: A number of real-time PCR assays for direct detection of MRSA in clinical specimens are targeting the SCCmec-orfX junction in the *S. aureus* genome. With respect to their implication as screening tests performed at admission of a patient at risk for MRSA infection, such assays should be as rapid and as sensitive as possible.

Selection of efficient primers and probes is complicated by the known sequence diversity among the various types of SCCmec cassettes. As a consequence, primer and probes are designed to cover the most common SCCmec types encountered in clinical MRSA isolates. Considering the enormous diversity of SCCmec sequences, rational primer selection can only be a best compromise between the coverage of as many SCCmec variants as possible and loosing analytical sensitivity due to primer multiplexing problems. Therefore incusivity rates may differ among the various PCR assay concepts.
Methods: Three commercial PCR tests targeting the SCCmec-orfX junction were evaluated for analytical sensitivity, time-to-result and for their performance to cover animal-associated MRSA strains of the clonal complex CC398 next to human epidemic MRSA strains.
Results: With exception of MRSA strains harboring "uncommon" SCCmec elements, all of the investigated human epidemic and animal-associated MRSA strains were detected by the Roche LightCycler MRSA Advanced Test, but the latter strains presented with a different Tm-value in melting curve analysis. DNA sequencing revealed single-nucleotide polymorphisms (SNPs) within the *S. aureus* orfX region characteristic for MRSA CC398 strains, which are obviously covered by the proprietary sensor hybridization probe of the assay. All MRSA strains covered by the LightCycler MRSA Advanced Test also tested positive in the BD GeneOhm MRSA and the Cepheid Xpert MRSA test, but either these real-time PCR tests had no option to perform Tm-analysis or viewing of melting curves.
Conclusions: As a practical application of the newly identified SNPs, we present the use of this commercial real-time PCR test for the direct detection and simultaneous identification of animal-associated MRSA strains. All of the investigated MRSA CC398 strains harbored at least one of the novel SNPs in the SCCmec-orfX junction – represented by a characteristic Tm of 55.5ºC in subsequent melting curve analysis. Since such a Tm-shift was not observed with any non CC398 MRSA strains yet, it may serve as a molecular marker for the presence of MRSA CC 398.

Antibacterial susceptibility testing and implications for resistance surveillance and treatment

S120 Problems with low level resistance against quinolones

L. Martínez-Martínez (Santander, ES)*

Bacterial resistance to quinolones is related to decreased permeability, active efflux, drug modification, target modification or target protection. Recent studies indicate that certain genes (recA, recC, tolC, fis, ruvC, xseA, xseB) also contribute to the intrinsic resistance of *E. coli* to quinolones. Microbiological and clinical aspects of low level quinolone resistance have been more often studied in enterobacteria. In these organisms, a single gyrA mutation causes resistance to nalidixic acid and MICs of fluoroquinolones as low as 0.125–0.25 mcg/ml (still higher than MICs of <0.008–0.06 mcg/ml for wild-type isolates). This mechanism favours the appearance of additional mutations in gyrA or in other topoisomerase-encoding genes, finally resulting in high level resistance. Target protection by Qnr proteins (often encoded by plasmid genes), decreased permeability by porin modifications or active efflux of chromosomal (particularly RND transporters) of plasmid (QepA proteins, OqxAB) origin also determine low level quinolone resistance. The Aac(6′)-Ib-cr enzyme modifies quinolones with a piperazinyl substituent. Both *in vitro* and *in vivo* data suggest that these additional low-level mechanisms may also represent an initial step for acquisition of increased resistance. Low level resistance mechanisms may be difficult to detect because they translate into MICs below current EUCAST or CLSI breakpoints. Interestingly, plasmid-mediated mechanisms may not sufficiently compromise the activity of nalidixic acid, which results in decreased susceptibility or low-level fluoroquinolone resistance in the absence of nalidixic acid resistance, which is in contrast with the usual consequences of mutations in topoisomerase genes. As quinolone efficacy is related to Cmax/CMI and AUC /CMI parameters, small variations (of even only 2–4 dilutions) due to low-level resistance mechanisms may contribute to therapeutic failure. Animal models and clinical data suggest a poor outcome of patients with severe infections caused by *Salmonella*, *Klebsiella* (and presumably other enterobacteria) with low level fluoroquinolone resistance. Because of these reasons, it would be important to know actual MICs of quinolones for clinical isolates, and to obtain additional information on quinolone resistance mechanisms. This will allow a better knowledge of risk factors for acquiring low level resistant strains, and a better definition of the actual therapeutic relevance of these organisms.

S121 Problems with low level resistance against glycopeptides

A. Soriano (Barcelona, ES)*

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains has complicated the treatment of *S. aureus* infections. Vancomycin is currently a cornerstone in the treatment of bacteraemia and infectious endocarditis (IE) due to this pathogen, but evidence linking vancomycin minimum inhibitory concentration (MIC) of 4–8μg/ml to a high failure rate of vancomycin has led to the susceptibility breakpoint being considered when the MIC is ⩽2μg/ml. However, vancomycin treatment failure is not uncommon even when MRSA strains are fully susceptible (MIC ⩽2μg/ml), and studies have described a reduction in vancomycin efficacy against MRSA strains with high MIC but within the susceptible range. The presentation will deal with the different methods for determining the vancomycin MIC and discuss the implications of understanding the relationship between vancomycin MIC and vancomycin efficacy.

Evolution and epidemiology of β-lactamases in Enterobacteriaceae

O127 Rapid increase of antimicrobial-resistant Gram-negative pathogens in Europe

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Objectives: *E. coli* is the most frequent cause of Gram-negative blood stream infections, and one of the most important food-born pathogens worldwide. *K. pneumoniae* has a central role in the acquisition of novel resistance mechanisms and may precede subsequent dissemination among other Enterobacteriaceae. Monitoring resistance in these pathogens is therefore important. In this study we determined the resistance proportions and the trends over time for invasive *E. coli* and *K. pneumoniae* infections in Europe.
Methods: Since 1999, EARSS (European Antimicrobial Resistance Surveillance System) has been collecting routine antimicrobial susceptibility testing data from seven major invasive pathogens. In 2008, almost 900 laboratories in 33 countries collected data from over 700,000 isolates. Resistance proportions were calculated for each participating country and trends in resistance over the past four years were calculated using the Cochran Armitage test (two-sided p-value <0.05).
Results: In 2008, four out of 33 (12%) countries reported *E. coli* fluoroquinolone (FQ) resistance below 10%, 18 (55%) countries between 10% and 25%, and 10 (30%) countries reported rates between 25% and 50%, and Turkey above 50%. FQ resistance increased significantly in 19 (58%) countries in the last four years. Although less then half of the countries (15/33) reported less than 5% resistance against 3rd generation cephalosporins (3GCs), resistance proportions have been increasing over the last four years in 21 countries. Two east European countries reported levels higher than 25%, Bulgaria (29%) and Turkey (42%). Combined resistance in *E. coli* was significantly increasing in 19 out of 33 (58%) countries.
Carbapenem resistance in *K. pneumoniae* is still absent in 21/31 countries, but seven countries reported rates from 1% to 5%, while in three countries this was much higher: Cyprus (10%), Israel (19%), and

Greece (37%). For combined resistance, the most frequent pattern was against all three classes of antibiotics (3GCs, FQ and aminoglycosides), 14%. The proportion of combined resistance from 9/31 (29%) countries increased significantly over the last four years.
Conclusion: The data gathered by EARSS over the years show an increasing loss of effective antimicrobial therapy. Combined resistance is the dominant threat imposed by invasive *E. coli* and *K. pneumoniae* in Europe. Delivery of crucial health services in hospitals and in the community is at stake.

O128 Emerging resistance mechanisms in ESBL-producing strains

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Objective: ESBL producing strains are increasingly reported to present additional resistance mechanisms. These multidrug resistant strains should be detected early to rationalize drug treatment and avoid increased selection of resistance. The aim of this study was to detect the presence of AmpC, carbapenemases and plasmid-mediated quinolone resistance (PMQR) (qnr, aac(6′)-Ib-cr and qepA) mechanisms in ESBL-producing strains by genotypic assays and compare their efficiency versus phenotypic methods.
Methods: ESBL- and AmpC-producing strains were identified by the double-disk test and double disk synergy test, respectively. Carbapenemases were phenotypically detected by the Hodge test. MIC of fluoroquinolones was detected by Etest. AmpC, carbapenemase, qnr, aac(6′)-Ib-cr and qepA genes were identified by multiplex PCR and sequencing. Topoisomerase II mutations were detected by sequencing of the quinolone-resistant determining region.
Results: In 2009, 200 ESBL-producing Enterobacteriaceae isolates were collected at the Microbiology Unit of the Padua Hospital. ESBL belonging to different classes (TEM, SHV, CTX-M and OXA) were characterized by genotypic analysis. Qnr and aac(6′)-Ib-cr genes were found in 26% and 8% isolates, respectively. Qnr was mostly present in *Klebsiella pneumoniae*, while aac(6′)-Ib-cr was found exclusively in *Escherichia coli*. QepA was not found. Both genes were localized on plasmids and could be both transformed and trans-conjugated in acceptor strains. MIC of fluoroquinolones on these acceptor strains indicated a 20–100 increased resistance due to the plasmid-mediated mechanism. However, high-level resistance to fluoroquinolone in the wild-type strains was due to the additional presence of topoisomerase mutations in strains presenting both ESBL and PMQR. AmpC were detected on 5.5% isolates of *Enterobacter* spp. and *Proteus mirabilis*. Carbapenemases were found in 3% isolates of *E. aerogenes, E. coli* and *K. pneumoniae*. Carbapenemases were subsequently genotypically characterized as IMP, VIM, OXA, KPC CMY or SME types.
Conclusions: Emerging resistance mechanisms were found in ESBL-producing strains, with PMQR being the most frequent. While genotypic assays implement phenotypic testing of AmpC and carbapenemases, they are the only methods available up to date for detection of PMQR. Hence, both phenotypic and genotypic methods should be employed to rationally direct the pharmacological treatment.

O129 Emergence of *Klebsiella pneumoniae* ST11-producing KPC-3 carbapenemase at a Lisbon hospital

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Background: KPC-type carbapenemases are emerging resistance in *Klebsiella pneumoniae* and other Gram-negative bacteria in several geographical areas since 2001. KPC producers area increasingly prevalent in USA, Israel and Greece, and have been recently detected in Asia, Latin America and Europe.
Objective: The aim of this study was to investigate the resistance mechanisms in a *Klebsiella pneumoniae* 2564FF clinical isolate resistant to oxyiminocephalosporins, α-methoxycephalosporins and carbapenems.
Methods: Minimum Inhibitory Concentrations (MICs) were determined by Etest technique and isolates were screened for extended-spectrum β-lactamases (ESBLs), metallo-β-lactamases (MBLs) and class A carbapenemase was detected by boronic acid assay. Polymerase Chain Reaction (PCR) was performed with primers designed to amplify blaSHV, blaTEM, blactx, blaKPC genes and respectively genetic environment. Population analysis by multilocus sequence typing (MLST) were screened by specific PCRs and sequencing analysis. M13 fingerprint typing method was used to compare this isolate with others *K. pneumoniae* isolates found in this hospital.
Results: *K. pneumoniae* 2564FF was isolated from a blood culture of a patient with leukemia at Santa Maria Hospital, Lisbon. The MIC for imipenem, aztreonam and to extended-spectrum cephalosporins was $\geqslant$32 mg/L. The resistant phenotype was unchanged by EDTA but the inhibition zones for imipenem, aztreonam and to extended-spectrum cephalosporins were increased with boronic acid. *K. pneumoniae* 2564FF produced a restrict β-lactamase SHV-1 and the carbapenemase KPC-3. The blaKPC-3 gene is part of a plasmid and no amplification was observed after PCR for detection of transposase, resolvase, and putative insertion sequences (IS) elements located upstream of blaKPC gene, suggesting that the blaKPC gene in *K. pneumoniae* 2564FF was included in a different genetic environment like Tn4401. M13 fingerprinting analysis revealed that this genotype had not been detected in the hospital previously. Also the sequence type ST11 was not detected in others *K. pneumoniae* KPC-producing strains described worldwide.
Conclusions: This is the first report of *K. pneumoniae* KPC-3 clinical isolate in Portugal. Our findings should alert medical authorities to implement stringent methods for the detection and spread control of emerging KPC carbapenemases.

O130 The changing epidemiology of carbapenem-resistant Enterobacteriaceae carriage in hospitalized patients

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Objective: *Klebsiella pneumoniae* (KP) ranks second as cause of Gram-negative sepsis at our medical center and forty five percent of the isolates display an extended spectrum β-lactamase producer (ESBL) phenotype. Starting in January 2006, the prevalence of carbapenem-resistant (CR) KP isolates increased significantly. Similar trends have been recorded worldwide.
In April 2006, an extensive infection control program was started in our medical center. In 2008 this program included surveillance to identify potential carbapenem-resistant Enterobacteriaceae (CRE) carriers among asymptomatic hospitalized patients being contacts of patients with KP-CR clinical infection and all patients admitted to the general ICU and to the hemato-oncology wards. The present study describes the results of this program.
Methods: Rectal swabs were obtained weekly and plated on KPC selective agar. Suspected colonies were identified by Vitek II system. Susceptibility testing for imipenem, meropenem, and ertapenem was performed using E-test (AB Biodisk) in accordance with the manufacturer's instructions. The molecular mechanism underlying resistance to carbapenems was assessed by PCR amplifiction of blaKPC and KP specific genes directly from bacterial isolates.
Results: During a 22-month period we assessed 8650 samples from 3239 patients (1810 patients in 2008 and 1429 in 2009). Of the 105 CRE carriers identified in 2008 (5.8% of patients tested), 100 (95.2%) were KP-CR carriers and 5 (4.8%) were CR *Enterobacter* sp. and/or *E. coli* sp. carriers. As the carrier rate decreased in 2009 (40 of 1429 tested patients, 2.8%; $p < 0.001$) the proportion of CRE carriers other than KP-CR increased significantly (11 of 40 positive patients, 27%; $p < 0.001$).
All of CRE isolates were found to carry blaKPC gene.
Conclusions: Our results show that during the present outbreak of CR-KP, a large number of hospitalized patients considered at risk are asymptomatic carriers and certainly constitute a major reservoir for the nosocomial spread of the organism. The presence of multiple CRE isolates suggests transfer of the genetic element encoding this β-lactamase between Enterobacteriaceae species in the gastrointestinal tract.

O131 Impact of EUCAST breakpoints on susceptibility levels of nosocomial Gram-negatives in Belgium

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Objectives: The implementation of EUCAST breakpoints was simulated on the susceptibility rates of isolates from 8 teaching hospitals in Belgium, collected from 1998 to 2007 as part of the MYSTIC study, against 5 antibiotics.
Materials and Methods: E-tests were used on 6993 unduplicated Enterobacteriaceae (EB) and 2409 *P. aeruginosa* isolates collected over a ten year surveillance study from patients admitted in ICU, hematology and general wards. Results were analysed using both CLSI and the new EUCAST breakpoints and compared.
Results: Among the EB (n = 6993), susceptibility rates according to CLSI and EUCAST breakpoints did not significantly decrease for meropenem (MEM); 99.6% and 99.1% respectively. A significant decrease in susceptibility rates ($P < 0.0001$) was seen for ceftazidime (CAZ): 83.8% to 75.5% ($P < 0.0001$), for cefepime (CPE): 96.9% to 88.0% ($P < 0.0001$), for pip/tazo (TAZO): 84.1% to 78.4% ($P < 0.001$) and for ciprofloxacin (CIP): 83.1% to 80.2% ($P < 0.0001$).
Susceptibility rates for *E. aerogenes* isolates (n = 766), among which TEM-24 and SHV-4 ESBL-producing isolates, decreased from 94.0% to 77.4% for CPE ($P < 0.0001$) and from 54.1% to 32.4% for TAZO ($P < 0.0001$) using CLSI and EUCAST criteria respectively; susceptibility rates for MEM did not decrease significantly and remained high at 96.3% and 94.2% respectively.
After implementation of EUCAST breakpoints, susceptibility rates of *P. aeruginosa* (n = 2409) for CAZ and CPE were not affected: 72.3% and 61.2% respectively. MEM remained the most active agent against *P. aeruginosa* though a decrease in susceptibility was observed from 81.6% to 75.2% ($P < 0.0001$). The activity of TAZO is affected the most with a decrease in susceptibility from 80.4% to 69.3% ($P < 0.0001$).
Conclusions: When implementing EUCAST breakpoints carbapenem activity against EB does not change whereas an important decrease in CAZ and CPE susceptibility rates are observed. MEM remains the most active agent against *P. aeruginosa* whereas the activity of TAZO is markedly decreased.

O132 Characteristics of the multi-resistant bacteria that actually diffuse in the Centre region, France, in and out of healthcare institutions

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Objectives: Epidemiological study involving the teaching hospital, 20 general hospitals, 7 local hospitals, 16 private clinics, 12 rehabilitation-care centers, 5 psychiatric clinics, 30 nursing homes (NHs) and 19 in town-clinical laboratories (for outpatients), to document the diffusion of multiresistant bacteria (MRB) into the region (2.8 millions unhabitants).
Methods: During 15 days, all MRB isolated from diagnostic clinical samples were documented and strains were centralized. After identification control, antibiotic susceptibility testing was performed (standard procedures). Genetic diversity of strains was studied (PFGE).
Results: The study involved 346,251 patient-days and 244,091 resident-days. During the study, 43,379 diagnostic clinical samples were performed: 7,564 blood cultures, 2,130 deep pus, 1,054 respiratory tract samples, 17,768 urines, 606 intravenous devices, 12,889 superficial pus. From the 43,379 clinical samples, 281 MRB were obtained (0.6%): 56% from urine (1.5% of urine samples), 23% from superficial pus (0.6% of pus), 9% from respiratory tract (5% of respiratory tract samples), 5% from deep pus, 4% from blood and one from intravenous devices. MRB belonged to 14 species. *S. aureus* (36%) and *E. coli* (31%) predominated, followed by *E. cloacae* (9%), *P. aeruginosa* (8%), *K. pneumoniae* (5%) and *A. baumannii* (2%). Vancomycin-resistant *E. faecalis* or *E. faecium* were not recovered. Diversity was the highest in healthcare institutions (HCIs). By contrast, *S. aureus* predominated in NHs, *E. coli* among outpatients. 70% of MRSA strains were non-multiresistant (usually named CA-MRSA). 79% of ESBL-producing *E. coli* strains were CTX-M. MRSA and ESBL-producing *E. coli* strains were (1) mostly resistant to fluoroquinolones (93 and 60% respectively), (2) genetically diverse and distinct from clones classically spreading into HCIs, (3) widely spread in HCIs, NHs and outpatients, (4) responsible for rare outbreaks, but in NHs. *E. cloacae, K. pneumoniae*, *P. mirabilis* and *A. baumannii* were the most involved in outbreaks into HCIs, and were associated with a lower genetic diversity.
Conclusion: MRD are unfrequent, but their diffusion is large, especially for recent MRSA and ESBL-producing *E. coli* clones.
Prevalence is the highest into HCIs, but MDR diffuse now out of HCIs, and especially in NHs.
We suggest that an effective alert system to detect emerging and/or epidemic phenomenon should involve MDR survey in HCIs, NHs and in town-clinical laboratories.

O133 Antimicrobial susceptibilities of Gram negative bacteria and antibiotic consumption in a Greek tertiary hospital, 2001–2008

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Objective: To examine differences in antibiotic susceptibility of Gram negative bacteria and in antibiotic consumption in a Tertiary Hospital, within 7 years of operation.
Methods: All clinical samples from inpatients. Identification and susceptibility testing, using the Wider® semi-automated system with CLSI breakpoints. Antibiotic consumption expressed as Defined Daily Doses (DDD) per patient-day.
Results: 1550 and 706 isolates identified during 2008 and 2001, respectively.
E. coli non-susceptibility rates were significantly higher in 2008 for Cefotaxime (12.5%; 69/551 isolates vs. 4.5%; 12/266 in 2001), Cefepime (14.9% vs. 3.8%); Cotrimoxazole (31.2% vs. 18.4%); Gentamicin (9.6% vs. 3.7%); and Ciprofloxacin (21.6% vs. 3.3%) (for all, $p < 0.01$).
K. pneumoniae non-susceptibility increased significantly for Cefotaxime (55.1%; 97/176 isolates in 2008 vs. 35.6%; 21/59 in 2001), Cefuroxime (58.5% vs. 31.8%); Cotrimoxazole (51.1% vs. 18.6%); Cefepime (53.4% vs. 5.1%); and Ciprofloxacin (51.7% vs. 13.2%) (for all, $p < 0.01$). *P. aeruginosa* non-susceptibility increased for Gentamicin (26.5%; 61/230 in 2008 vs. 14.2%; 15/95 in 2001, $p = 0.01$), Amikacin (18.2% vs. 13.1%), Pip/Tazo (15.2% vs. 10.9) and Cefepime (25.6% vs. 16.3%). In 2008, non-susceptibility rates to anti-pseudomonal antibiotics ranged from 18.2% (Meropenem) to 26.1% (Ciprofloxacin). *A. baumannii* non-susceptibility increased significantly for Pip/Tazo (82.2%; 180/219 vs. 37.8%; 17/45, $p < 0.01$). In 2008, non-susceptibility rates ranged from 52.1% (Cefepime) to 88.6% (Ciprofloxacin).
Pan-Drug Resistant (PDR − to B-lactams, Carbapenems, Amikacin, Ciprofloxacin and Colistin) bacteria isolated in 2008: *Klebsiella* (12), *A. baumannii* and *P. aeruginosa* (1 each). No PDR isolates were found in 2001.
Consumption of antibiotics with activity against Gram negatives increased by 30.3% (15.9 DDD/100 hospital-days in 2008 vs. 12.2 in 2001). Increase was more marked for Carbapenems (3.2 vs. 1.1; $p < 0.03$), Fluoroquinolones (6.4 vs. 4.3) and Colistin (0.05 vs. 0.03), whereas decreased was the consumption of 3rd Generation Cephalosporins (2.1 in 2008 vs. 2.3 in 2001) and the anti-pseudomonal penicillins (3.8 vs. 4.0).
Conclusion: Non-susceptibility rates of Gram negative bacteria markedly increased within 7 years and PDR strains emerged. This paralleled the increased consumption of wide-spectrum antibiotics (mainly Carbapenems and Fluoroquinolones) and of antibiotics used as last-resource (Colistin).

O134 Recent epidemic emergence of blaNDM-1 metallo-β-lactamase in enteric organisms from India is mostly linked to A/C plasmids

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Objective: Metallo-β-lactamases are powerful resistance mechanisms that can generate insensitivity to virtually all β-lactam antibiotics. The recent epidemic emergence of this resistance mechanism in *Klebsiella* spp., *E. coli*, *Enterobacter cloacae*, *Citrobacter freundii* and *Providencia rettgeri* in geographically distant areas of India and the UK is a major cause for concern. This project was initiated to determine the genetic vehicle(s) responsible for the rapid emergence and dissemination of the blaNDM-1 MBL in enteric organisms.
Methods: Species identification and antibiotic susceptibility profile of 67 blaNDM-1 harbouring enteric isolates collected from Chenai (south India) and Haryana (North India) was performed using the Phoenix machine. Isolates were typed by pulsed field Gel electrophoresis after Xba1 digestion. Plasmids were visualized by ethidium bromide staining following PFGE of S1 partially digested plugs. Plasmids carrying blaNDM-1 were further identified by probing of the same gels using a 32P labelled blaNDM-1 and A/C rep probes. Transconjugants were selected following matting with J53 on MacConkey plates containing azide and 0.5 mg/L meropenem. Plasmid analysis on A subset of transconjugants (20) was performed by replicant typing and S1 PFGE and subsequent probing. A/C plasmid analysis was further performed using a set of 12 primers designed to amplify sections of the backbone of A/C plasmids and DNA probing with A/C probes.
Results: All isolates from the North of India were *Klebsiella pneumoniae* and carried A/C plasmids of size 50kb (9), 80–90kb (2), or 118kb (11). However, in the south of India isolates were of various enteric species and gave numerous different pulsed field gel restriction types. Furthermore plasmids found in the isolates from the south were of many different sizes ranging from 100kb to 375kb in size. Probing indicated that most plasmids carrying blaNDM-1 were of rep A/C type (19/20) only 1/20 tested were found to be carried on the F plasmid. Transconjugants were selected for all strains except the small plasmids from the North of India. Transconjugants gave plasmids of mostly the same size but examples were found of size changes following transconjugation including both increasing and decreasing size. PCR on transconjugants and parents indicated differences in the A/C plasmid backbone.
Conclusions: blaNDM-1 emergence in enteric organisms in India is linked mostly to A/C plasmid types of varying sizes ranging from 50kb to 375kb.

O135 Stabilization in prevalence of ESBLs and ciprofloxacin non-susceptibility in Enterobacteriaceae from blood in the United Kingdom and Ireland

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Objective: *E. coli* is the most frequent cause of bacteraemia in the UK, and increasing antimicrobial resistance could severely compromise current therapeutic approaches. The BSAC Bacteraemia Resistance Surveillance Programme has monitored resistance in pathogens from blood, including Enterobacteriaceae, since 2001.
Methods: Each year, 25 laboratories in the UK and Ireland sent up to 10 isolates each of *E. coli* (20 in 2008), *Enterobacter*, *Klebsiella*, Proteeae and other Gram-negative bacteria for central MIC testing by BSAC methods. The presence of ESBLs was deduced phenotypically from clavulanate synergy and blaCTX-M genes were sought by PCR.
Results: The prevalence of ESBL production in *E. coli* and *Klebsiella*, and ciprofloxacin non-susceptibility in *E. coli*, rose markedly in the early 2000s, but has not risen further since 2006. Segmented logistic regression (assuming two periods) located change points near early 2006 for *E. coli* and early 2004 for *Klebsiella*, with no significant increase in ESBLs or ciprofloxacin non-susceptibility in the later period. Prevalence of resistance, including AmpC and ESBLs, has changed little in *Enterobacter* over this period, and ESBLs remain very rare in Proteeae and *Serratia*. ESBL production increased with similar time course in both infections acquired in hospital (>48 hours after admission) and others, but was always more prevalent in hospital infections (twice as prevalent for *E. coli* and *Enterobacter*, three times for *Klebsiella*, 2005–08.) CTX-M ESBLs were predominant, comprising 90% of ESBLs in *E. coli* and 67% in *Klebsiella* but only 22% in *Enterobacter* in 2005–08. ESBL producers were commonly multiresistant, as previously noted. In 2008, all ESBL-producers remained susceptible to imipenem, meropenem and doripenem, although a few non-producers (1 *Klebsiella*, 3 *Enterobacter*) were non-susceptible to one or more of these agents. The spread of multiresistant ESBL-producers did not explain all the changes seen in antimicrobial resistance: among *E. coli* (but not other genera) ciprofloxacin non-susceptibility in ESBL non-producers also increased from 2001, and appears to have stabilized from 2007, standing at 12% in 2008.
Conclusion: Prevalence of ESBLs in *E. coli* and *Klebsiella*, and ciprofloxacin non-susceptibility in *E. coli*, rose rapidly in the early 2000s. It has now stabilized, but at a level that demands continued vigilance and care in the selection of empirical treatment.

ESBL production and ciprofloxacin non-susceptibility: % of isolates from blood, 2001–2008

Year	*E. coli*			*Klebsiella*			*Enterobacter*		
	N	ESBL	CIP	N	ESBL	CIP	N	ESBL	CIP
2001	245	0	8	233	6	10	180	6	13
2002	250	3	7	242	5	15	206	9	17
2003	248	2	11	245	8	9	217	6	16
2004	248	6	18	225	16	18	206	9	13
2005	247	7	17	237	13	16	213	15	14
2006	242	12	26	237	13	15	198	10	17
2007	248	9	24	227	12	18	204	9	17
2008	467	9	19	206	12	15	157	9	13

ESBL = % producing ESBL; CIP = % non-susceptible to ciprofloxacin (MIC $\geqslant$1 mg/L).

O136 Risk factors for intestinal carriage of Enterobacteriaceae with extended-spectrum β-lactamase-producing phenotype on a medical ward

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Background: Intestinal carriage is a reservoir for invasive infection with ESBL producing Enterobacteriaceae (ESBL PE) and their dissemination. The aim of the study is to establish the risk factors for intestinal carriage in patients admitted on a medical ward with infectious and noninfectious diseases.
Method: A prospective study conducted between 1st Aug and 31st Oct 2009 on patients hospitalized for more than one week. Patients were screened on discharge (convenience sample). Rectal swabs were placed in 1 ml sterile 0.9% saline then inoculated on cultured media Chrom ID ESBL Biomerieux. The results were confirmed with double disc diffusion assay according to CLSI. Univariate and multivariate analyses were performed.
Results: 153 patients were screened (46% male, median age 53.8 years) from which 129 patients (84%) were admitted for infectious diseases. 64 ESBL PE were isolated (carrier rate 41.83%). ESBL PE included: *E. coli* (39), *Klebsiella* spp. (21), *Proteus* spp. (2), *Serratia* spp. (2). Variables associated with ESBL PE carriage by univariate analysis: length of stay ($p = 0.03$), length of antibiotic use ($p = 0.02$), use of ceftriaxone ($p < 0.01$), use of ciprofloxacin ($p = 0.003$). In multivariate analysis, only length of stay ($p = 0.004$) and use of ciprofloxacin ($p = 0.001$) were independently associated with carriage of ESBL PE.

Conclusions: ESBL PE carriage rate after hospitalization and treatment in our medical ward is high. Length of stay and use of ciprofloxacin are important risk factors for intestinal carriage with ESBL PE.

Clinical mycobacteriology

O137 Risk factors of death among hospitalized patients with tuberculosis: a report from Iran

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Background: Although mortality levels have decreased there is still significant number of people, especially in Iran, expiring as a result of TB infection. In this study we evaluate risk factors of death among hospitalized patients with TB.
Method: The retrospective study was carried out at the National Research Institute of Tuberculosis and Lung Disease (NRITLD) in Tehran, Iran from May 2003 to Dec 2009. Patients with documented pulmonary TB were included. A death case is considered as a case that died during the treatment of TB from any causes. Data was gathered from medical records and compared between two groups of patients who died and the group who didn't die during their treatment. These variables include age, sex, nationality, history of smoking and opium, HIV status, history of TB treatment, co-morbidity, symptoms and sign of TB, cavitary lesion, and other demographic, clinical and radiological factors. All data were entered into SPSS (Version 15.0). The Cox-proportional hazard model was used for the significant factors with survival.
Results: In this study, 1897 tuberculosis patients were included. The mean age was 50.18±21.13 years and 54±20 in all patients and in death group respectively, a difference that was statistical significant. 973 were male (51.3%).76.8% of the patients were Iranian and the remaining 440 (23.2%) were from the neighboring countries. During the hospitalization, 163 (8.6%) patients died. The mean duration of admission was 34±36.8 days (Median = 22 days, Range 1–365). Totally among whom HIV test was performed, 32 (22.2%) cases of the death group and 82 (14.3%) of the control group were found to be HIV positive that reach statistical significance.
The mean duration of symptoms to admission was 8.27±16.79 months. Notably, cough, dyspenea, and fever were more commonly seen in death group ($P < 0.05$).
During the course of treatment, 231 (12.2%) developed drug-induced hepatitis that 45 (19.5%) of the patients died ($p < 0.05$). In χ^2 analysis, male sex, Iranian nationality, opium addiction, HIV positive status, concomitant respiratory and immunosuppressive diseases and MDR-TB were statistical significant in death group.
In the stepwise Cox regression model, is showed that TB history, smoking, co-morbidity, involvement of lung, cavitary lesion, Drugs induced hepatitis, WBC < 4000 and >10000 and age >65 affected the in-hospital mortality rate (Table 1).
Conclusion: Identifing these risk factors may improve clinical outcome of patients.

Table: Cox-proportional hazard model of factors

	Multivariate logistic regression		
	Crude HR	HR	95% CI
TB history	2.270	1.562	1.023–2.383
Smoking	0.538	0.621	0.443–0.871
Co-morbidity	0.326	0.424	0.295–0.610
Involvement of lung	0.899	0.498	0.311–0.798
Cavitary lesion	1.650	1.379	0.960–1.980
DIH	2.155	1.592	1.112–2.282
WBC	1.425	2.169	1.555–3.027
Age >65	1.896	1.688	1.165–2.445

O138 *Mycobacterium* in paediatric patients during a 2-year period in a health district of Madrid, Spain

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Objective: The aim of this study is to describe the prevalence of tuberculosis and non-tuberculosis mycobacteria isolates in paediatric patients during a two year period (November 2007 to November 2009) in a health district of Madrid (Spain).
Methods: Four hundred and ninety eight specimens from paediatric patients were proceseed for mycobacterial study. All of them were prepared for auramine stain and decontaminated by NaOH-N-acetylcysteine method. When the specimen was a gastric acid, it was neutralizated before with bicarbonate of sodium. After decontamination each specimen was cultured in liquid and solid medium (MIGT and Coletsos, respectively). If growth was detected a Zielh-Neelsen stain was done. Accuprobe kit (Gen-Probe) was used to identificate *Mycobacterium tuberculosis* Complex or *Mycobacterium avium* Complex strains. Other Non Tuberculosis Mycobacteria (NTM) were identified by solid hybridization using GenoType *Mycobacterium* kit (Hain Lifescience).
Results: Thirty two (6.42%) out of 498 specimens received were positive: 17 *Mycobacterium tuberculosis* Complex (53.13%), 9 *Mycobacterium abscessus* (28.12%), 4 *Mycobacterium avium* Complex (12.5%) and 2 *Mycobacterium lentiflavum* (6.25%).
They were obtained from different specimens from 22 patients:
– *M. tuberculosis* Complex: 14 gastric juice, 2 pleural liquid and 1 sputum.
– *M. abscessus:* 7 sputum, 2 bronchoalveolar lavage.
– *M. avium* Complex: 2 fine needle aspiration biopsies (FNAB), 1 gastric juice and 1 bronchoalveolar lavage.
– *M. lentiflavum:* 2 FNAB
Five out of 22 patients were cystic fibrosis patients, in 4 of them the microorganism isolated was *M. abscessus.* In the other one, *M. lentiflavum* was recovered.
Conclusions: The most common specimens received were those of the respiratory tract, where *M. tuberculosis* was the most prevalent mycobacteria followed by *M. abscessus.* On the other hand in FNAB specimens the species isolated were *M. avium* Complex, and *M. lentiflavum. M. abscessus* is the most common mycobacteria isolated in cystic fibrosis patients in this population during this period.

O139 Seroprevalence of human immuno-deficiency virus infection among patients diagnosed with sputum smear positive pulmonary tuberculosis at an infectious diseases hospital, Kano, Nigeria

Y. Mohammed (Kaduna, NG)*

Objective: The main objective of the study is to estimate the HIV seroprevalence among patients diagnosed with sputum smear positive pulmonary tuberculosis (PTB).
Methods: Inorder to estimate the HIV seroprevalence one thousand six hundred and ninety-two (1,692) males (1066) and females (626) patients aged 15 years and above, with no previous TB treatment that presented to the chest clinic with symptoms like cough, night sweats, fever, weight loss, chest pain etc, and whose initial sputum smears demonstrated acid fast bacilli (AFB) by direct smear sputum microscopy using Ziehl-Nelseen (ZN) stain at least two specimens in line with WHO recommendation. Each patient was offered confidential HIV testing accompanied by pre and post-test counseling accordingly. Those that agreed to be screened for HIV antibodies had blood sample taken for the test and performed according to the standard hospital practice and followed guidelines developed by the National HIV Rapid Test Algorithm using ELISA Test of Capillus, Genie 11 and Determine HIV kit.
Results: The overall HIV prevalence was 38%, of that value, male, accounted for 37% and females 41%. There was no statistical difference. However, the prevalence is 48% in the age group 25–34years as

compared to only 12% in the age group 55–64years which was statistically different ($P < 0.05$). A higher proportion of females (42%) than males 37% were HIV Seropositive, but the difference is not statistically significant ($P > 0.05$).
Conclusion: There is high Human Immunodeficiency Virus prevalence (38%) among the studied patients with sputum smear positive pulmonary tuberculosis. The link between HIV and TB is often well known but few TB patients currently have the opportunity to know their HIV status and the stigma attached to HIV may deter people with features of suspicious of TB from seeking TB care.

O140 Different clinical isolates of *Mycobacterium tuberculosis* induced distinctive pulmonary inflammation in mice

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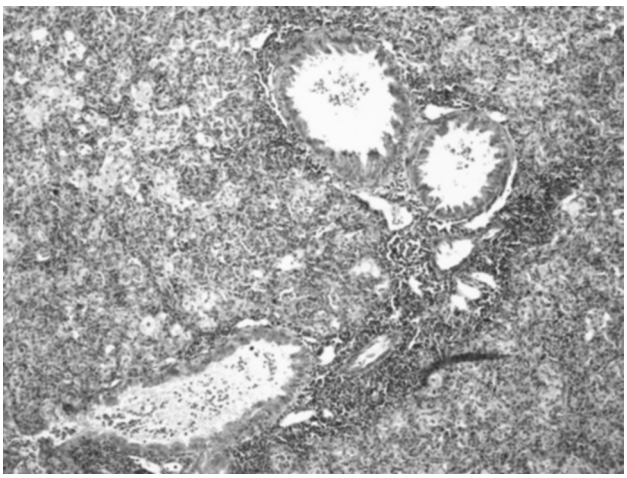
Background: *Mycobacterium tuberculosis* (Mtb) is a virulent intracellular pathogen that infects and persist in host macrophages, resulting in granuloma formation and collagen deposition in the lung. The mechanisms that confer resistance to Mtb or results in establishment of disease are poor understood. Data from the literature suggest that differences in Mtb virulence contribute to setting up of the disease. In order clarify this aspect, our purpose is to investigate the immune response and lung pathology in mice infected with Mtb obtained from distinct clinical isolates. The isolates were recovered from patients with noncavitary (SV 009) or extrapulmonary (SV 068) active tuberculosis.
Methods: Female Balb/c mice were infected intratracheally with 1×10^5 CFU/100 μL of Mtb clinical isolates. Neutrophils and mononuclear cells recruitment to the lung were accessed by bronchoalveolar lavage at 30 days post infection and lung histology were evaluated on 30 and 60 days post infection.
Results: Mice infected with SV 068 showed 22% more neutrophils (9×10^5/mL) and 70% more mononuclear cells (6×10^5/mL) recruited to bronchoalveolar space 30 days post infection, when compared with mice infected with SV 009, 5×10^5 mL and 4.5×10^5 mL respectively. The histology analysis of lung tissue, demonstrated that animals infected with SV 068 present greater number of foamy macrophages containing aggregations of Mtb, especially at 60 days post infection. Also, in this period, we observed the presence of more intense infiltrate of neutrophils in perivascular and perialveolar spaces when compared with animals infected with SV 009.
Partial Conclusion: Our preliminary findings suggest that the host defense can vary accordingly to the type of clinical isolation, leading to a correlation between the virulence and the source of infection.



O141 Infection of a knee joint with *Mycobacterium avium* in a patient with sarcoidosis

J. Hahne, T. von Stein, M. Militz, R. Werle, V. Bühren (Murnau, DE)*

Objective: Infections of osteoarticular tissue caused by *Mycobacterium avium* are reported in the literature but are still rare. *Mycobacterium avium* is found throughout the environment, the transmission is still unknown. Patients show a variety of symptoms and the septic process is insidious which is leading to a delay of diagnosis. Most patients reported on were immunocompromised. Potential treatments are either surgery, antituberculous drugs or both.
Method: We present a case of a 33 year old Caucasian male with a *Mycobacterium avium* infection of the knee joint.
Results: In March 2009, a 33 year old Caucasian male with previously documented sarcoidosis presented in a german university hospital with pain and swelling in the right knee joint. The patient underwent knee arthroscopy and consequetively developed purulent knee arthritis and a lateral joint fistula. Repetitive debridements were performed. The histological examination of the resected tissues showed granulamatous inflammation. *Mycobacterium avium* was confirmed via culture. In addition to surgery the patient was treated with ethambutol, clarithromycin and rifabutin. The fistula persited and therefore treatment was changed to air dressing. At the request of the patient he was sent to our specialist department for septic surgery. Repetitive debridements were started. Tissue samples revealed *Pseudomonas* aerogenosa and *Staphylococcus haemolyticus*. Tests for *Mycobacterium avium* were positive. Because of superinfection with the listed bacteria ethambutol was replaced by ciprofloxacin. The soft tissue quality improved. In august 2009 a ALT flap was transplanted. Postoperative progress was uncomplicated – the patient was discharged in September 2009. We recommended treatment with clarithromycin, ciprofloxacin and rifabutin for another 7 months. Mobilisation at full weight bearing as pain allowed was started at discharge. Definitive treatment options have to be reevaluated 6 months after last surgery. A total knee replacement in the case of negative microbiological tests is optional.
Conclusion: In immunocompromised patients with osteoarthritis the infection with ATM (atypical *Mycobacterium*) should always be considered. Treatment should include both: surgery and antituberculous drugs. Knowing the *in vitro* resistance of most ATM against antituberculous drugs, it's debatable whether the superinfection of other bacteria is clinically relevant for the symptoms and should be treated with priority.

O142 *Mycobacterium conceptionense (M. fortuitum* group) catheter-related bloodstream infection in an immunocompromised patient – necessity for antibiotic treatment after removing the central venous catheter? Review of literature

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Objective: Atypical Mycobacteria can cause disease in both healthy and immunocompromised individuals. They can rarely cause disseminated infections. Nevertheless infections in immunocompromised patients by atypical mycobacteria are described.
Results: We report a case of a 54-year-old man with diffuse large b-cell lymphoma (DLBCL) stadium III E B of the stomach, proximal duodenum, axillary, inguinal and cervical lymph nodes after six cycles R-CHOP 14 and one cycle R-DHAP chemotherapy received via central venous catheter (CVC) who develops leucopenia and anemia. Blood culture reveals *Mycobacterium fortuitum*. On admission to our hospital several days later a blood sample reveals leucocytosis (10.97×10^9 cells/litre), low hemoglobin level (9.8 g/dl) and thrombocytosis (517×10^9 platelets/litre). The C-reactive protein level was 43.9 mg/l. The patient has no fever. Blood cultures taken simultaneously peripherally and via CVC reveal both *Mycobacterium conceptionense* (*Mycobacterium fortuitum* group). The CVC is removed, but routine culture is negative for Mycobacteria. Because of a sustained bacteraemia for more than ten days and an underlying

immunocompromission antibiotic treatment with clarithromycin (500 mg twice daily) and amikacin (15 mg/kg once daily) is done for two weeks in accordance with the guidelines of the American Thoracic Society (ATS). A repeat blood culture after therapy is sterile.
Conclusion: In literature there are only few descriptions of invasive infections with rapidly growing *Mycobacterium fortuitum*. In a setting of underlying immunocompromised disease we treat bloodstream infection, advocate a duration of two weeks after having removed the catheter and review literature for the necessity for antibiotic treatment.

O143 Human *Mycobacterium bovis* infection in Buenos Aires: epidemiology, microbiology and clinical presentation

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Objectives: Describe the clinical, epidemiological, and microbiological characteristics of a series of cases of *M. bovis* infection in humans diagnosed in the Infectious Diseases 'Francisco J. Muñiz' Hospital, Buenos Aires, Argentina.
Methods: Analytic and retrospective study of clinical, epidemiological and laboratory findings of 39 patients with confirmed diagnosis of *M. bovis* infection (1996–2008).
Results: N = 39. Male: 28/39 (72%). Age (median): 45 years. Previous conditions: Diabetes 8/30 (27%), HIV(+) 8/37 (22%) (CD4 count (median): 34 cells/mm^3), malignancy 1/30 (3%). Domicile: Buenos Aires city and Conurbano: 22/22 (100%).
Risk factor for *M. bovis:* 26/28 (93%); a) Occupational exposure: 17/26 (65%) (meat processing plant (n = 9); butcher's shop (n = 4); tannery (n = 2); handling meat for pets (n = 2)); b) Consumption of unpasteurized milk 1/26 (4%); c) History of living in rural area: 8/26 (31%).
Clinical presentation: fever 16/25, malaise 14/25, weight loss 20/25, night sweats 8/25, cough 21/25, expectoration 21/25, haemoptysis 7/25, dyspnea 7/25, headache 2/25. Pulmonary disease: 29/39 (74%), extrapulmonary disease: 4/39 (10%); both: 6/39 (15%). Extrapulmonary disease included: lymph nodes 5/10, skin 1/10, bone and joints 2/10, renal 2/10, CNS 2/10. Comparing risk factor for *M. bovis* and pulmonary disease: occupational 17/17 (100%) vs. non occupational 6/9 (67%) (p = 0.03). Chest X-ray: cavities 10/26, miliary 2/26, pleural effusion 2/26, alveolar infiltrates 13/26. Mantoux test (+): 6/17 (35%). Outcomes: died 5/22 (23%) (HIV(+) = 3/3) (p = 0.01). Microbiology results: pulmonary specimens (sputum=35, BAL=2): Ziehl–Neelsen stain (ZN) (+): 28/37 (77%); Lymph nodes (n = 5): ZN(+): 1/4 (25%). All samples grew well in Stonebrink medium and neither in Lowenstein-Jensen. Susceptibility: Pirazinamide (Z) resistance (natural): 39/39. Susceptibility to isoniazid (H)–rifampin (R)–ethambutol (E)–streptomycin: 27/29 (93%). R resistance: 1/29 (3%). Multidrug resistance (H+R): 1/29 (3%). Empirical treatment (H–R–E–Z): 25/27 (93%). Follow up sputum (after day 60): ZN(+): 3/9.
Conclusions: The most important risk factor was occupational exposure. Pulmonary disease, indistinguishable from tuberculosis, was the most frequent clinical presentation. A high proportion of the patients had a compromised immune system with a high mortality among HIV(+) patients. In contrast to what is commonly described the most likely route of infection in our series was airborne transmission.

O144 Features of non-tuberculous mycobacterial pulmonary infections in an eight-year cohort of non-HIV-infected patients

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Background: Non-tuberculous mycobacterial (NTM) pulmonary infections predominantly affect patients with advanced HIV and those with chronic lung disease.. Data on the nature of infection, treatment course and outcomes in the UK are sparse.
Objectives:

- To identify and characterise NTM pulmonary infections with slow growing mycobacteria in non-HIV patients, diagnosed within a south London teaching hospital between 2000 and 2007
- To assess underlying risk factors for infection
- To assess treatment given, incidence of side effects, treatment cure and relapse rates

Methods: In this retrospective study 2000–2007, we examined pulmonary NTM infections south London hospital. Cases were identified through the microbiological database and case notes records. Inclusion criteria were: culture positivity for *Mycobacterium kansasii* (MK), *Mycobacterium avium* intracellulare (MAI), *Mycobacterium xenopi* (MX) and *Mycobacterium* malmoense (MM); >18 years old; HIV negative; meeting the ATS (American Thoracic Society) clinical criteria. Radiological reports were made by a radiologist. Statistical analyses were made on SPSS v15, using t tests and Mann Whitney U as appropriate.
Results: Of 211 patients identified with NTM isolates, 57 met inclusion criteria. Males (76%) with a median age of 60.5 years predominated. Predisposing factors were smoking (76.1%), alcohol (30.4%) and COPD (39.1%). Radiologically, cavitation (63.6%) and pulmonary infiltrates (31.8%) were common findings. 47 patients were treated, 10 not treated and 5 died. The predominant organism was MK, 35 of 47 cases (74.5%). Of the MK infections, there was a 100% cure rate with a 10% relapse rate over a 3 year follow up period. Treatment courses differed from British Thoracic Society (BTS) guidelines, most patients receiving triple therapy of rifampicin, ethambutol and usually clarithromycin or ciprofloxacin for >9 months. Side effects were common, occurring in 30% of treated infections. The statistically significant predictors of a poor outcome were a low pre-treatment weight and absence of fever.
Conclusions: *M. kansasii* is the predominant NTM organism in London. Optimal treatment regimens are unclear. In this study many patients were treated with 3 agents, in counterpoint to BTS guidelines. However, adding a third agent to the treatment regimen for MK did not appear to reduce the relapse rate, but did increase the risk of side effects.

Table. Outcome of patients with *M. kansasii*

	Treatment success N = 25 (%)	Relapse/Died N = 8 (%)	p value
Baseline characteristics			
Median age (range)	58 (25–87)	7.5 (59–80)	0.081
Male sex	18 (72)	5 (62.5)	0.652
Median Pre-treatment weight (range)	60	47	0.046
Median weight difference, kg	+1.4	–0.1	0.517
Symptoms & predisposing factors			
Weight loss	11 (44)	5 (62.5)	0.394
Haemoptysis	4 (16)	3 (37.5)	0.304
Fever	2 (8)	0	0.022
Alcohol	7 (28)	2 (25)	0.876
Prior rnycobacterial disease	2 (25)	3 (12)	0.48
COPD	8 (32)	3 (37.5)	0.795

O145 Absence of non-tuberculous mycobacteria recovery in sputum of cystic fibrosis patients despite adequate decontamination: a possible role of specific antimicrobial therapy used in our centre

E. Andre, J. Degraux, A. Simon, A. Ferroni, T.D. Huang (Brussels, BE; Paris, FR)*

Objectives: Non-tuberculous mycobacteria (NTM) were pathogens of growing importance in non-paediatric cystic fibrosis (CF) patients. In our centre, the prevalence of NTM in these patients observed (0.5%) during the last decade was markedly inferior to those reported in the literature ranging from 6.5% to 24%. The aim of this study was to screen for NTM in adult patients in our centre with 3 different decontamination methods for mycobacterial cultures in order to determine whether the choice of the decontamination technique of samples may have an impact on their recovery in our centre.
Methods: Between January and June 2009, consecutive sputum samples from adult patients with clinical suspicion of NTM infection (respiratory function degradation without other microbiologic explanation) were included in this study. 3 different decontamination protocols were

used: N-acetyl-l-cysteine (NALC)-NaOH (DCT1), NALC-NaOH-oxalic acid 5% (DCT2) and DTT-Chlorhexidine 1% (DCT3). Decontaminated specimens were than cultured onto both solid Loewenstein-Jensen slants (LJ; BioRad) and liquid MGIT (BD) culture. A sample was considered NTM negative if no NTM isolate was recovered after a standard 42-day incubation protocol.
Results: 36 sputum samples were collected from 26 adult patients during the study period. The median age of patients was 26 years. 88% of the patients received antimicrobial active against mycobacteria for more than 24 months before sputum collection (61% of the patients had fluoroquinolones and 88% had macrolides). No NTM was detected in the 36 specimens with none of the three decontamination techniques.
Conclusions: Long-term use of macrolides and fluoroquinolones may be an explanation for the absence of recovery of NTM in these CF patients. Further studies in other CF centres prescribing similar antimicrobial treatment regimens are warranted to support this observation.

O146 *M. haemophilum* outbreak among 9 Swiss women after permanent make-up of the eyebrows

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Objective: Between May and September 2009 9 patients were referred to our infectious diseases outpatient clinic because of skin lesions and suppurative lymphadenitis after permanent make-up of the eyebrows. We present the clinical and microbiological characteristics of the patients and the results of the outbreak investigation.
Methods: All but one patients had at least a lymph node fine needle aspiration, whereas skin biopsy of the eyebrow was performed only in 4 cases. Microscopic examination for mycobacteria, culture and broad-range mycobacterial PCR (16 S rRNA) were performed. Diagnosis of the first case was established using broad-range mycobacterium PCR. As PCR was positive for *M. haemophilum,* special culture was performed. After notification to the public health authorities, an investigation was started and all possibly contaminated material was examined for mycobacteria.
Results: All patients presented with an inflammatory lesion of the eyebrow associated with ipsilateral lymphadenitis located within the parotid gland. The lesions appeared 2–6 weeks after permanent make-up of the eyebrows. Fine needle aspiration and skin biopsy of the eyebrow were positive for mycobacteria in 8 and 1 case, respectively. Microscopic examination showed acid-fast bacilli in 6 cases whereas broad-range PCR was positive for *M. haemophilum* in 5 cases. Culture was positive for 7 patients after 14–70 days (median 44 days) and the microorganism was identified as *M. haemophilum.* All patients were treated with various combinations of ciprofloxacin, clarithromycin and either rifampin or rifabutin. Parotidectomy, local eyebrow excision and partial neck dissection were performed in 3 cases. Three patients were in complete remission following surgical intervention, one patient was in partial remission after two months of conservative treatment. The remaining 5 patients were stable after 2–6 weeks of antibiotic therapy (still ongoing). Outbreak investigation showed that all make-up procedures were performed by the same tattoo artist. PCR of the the make-up ink was positive in 6 out of 19 samples for *M. haemophilum,* cultures are still negative after 5 weeks.
Conclusions: We report the first *M. haemophilum* outbreak after permanent make-up due to contamination of the make-up ink. PCR analysis of mycobacterial 16S rRNA is an effective tool for early diagnosis and for appropriate selection of culture method. Optimal management of the infection requires further studies.

Vaccines: from Petri dish to populations

O147 Vaccination with *Acinetobacter baumannii* outer membrane proteins elicits protective immunity against multidrug-resistant and pan-resistant strains

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Objective: Over the last two decades the incidence of hospital-associated infections caused by multi-drug resistant *Acinetobacter baumannii* has increased significantly, requiring the development of novel approaches for controlling this infection. The objective of the present study is to develop a prophylactic vaccine for the prevention of *A. baumannii* infection.
Methods: Outer membrane proteins (OMPs) were purified from *A. baumannii* strain ATCC 19606, combined with an aluminium adjuvant, and used to vaccinate C57BL/6 mice by intramuscular injection. Levels of anti-OMP antibodies in serum were quantified by ELISA, and the ability of serum to prevent bacterial adherence to the A549 epithelial cell line was determined. Mice were challenged with the ATCC 19606 strain and 2 clinical isolates using a disseminated sepsis model, and the following parameters were measured: i) bacterial loads in tissues, ii) serum levels of pro-inflammatory cytokines, and iii) mortality. The ability of serum from vaccinated mice to protect naive mice from infection by passive transfer was determined.
Results: Two doses of the vaccine three weeks apart elicited high levels of OMP-specific antibodies which were able to block bacterial adherence to A549 cells. At 12 hours post-challenge, vaccinated mice had fewer bacteria than control mice in spleens (3.78 vs. 9.07 log10 cfu/g; $p < 0.001$), kidneys (3.50 vs. 8.33 log10 cfu/g; $p < 0.001$), and lungs (3.73 vs. 8.95 log10 cfu/g, $p < 0.001$). Vaccinated mice had lower serum levels of the pro-inflammatory cytokines IL-1β (2.0 vs. 396.1 pg/ml, $p < 0.001$), TNF-α (59.3 vs. 351.9 pg/ml; $p = 0.002$), and IL-6 (188.6 vs. 51141.3 pg/ml; $p = 0.007$) than control mice 12 hours post-challenge. Vaccinated mice had increased survival over control mice after challenge with the ATCC 19606 strain (100% vs. 0% survival; $p < 0.001$), a multi-drug resistant clinical isolate (70% vs. 10% survival; $p = 0.007$), and a panresistant clinical isolate (100% vs. 10% survival; $p < 0.001$). Mice passively immunized with serum from vaccinated mice 3 hours prior to challenge were protected from infection, whereas control mice receiving non-immune serum were unprotected (100% vs. 0% survival; $p = 0.003$).
Conclusion: Immunization with an OMP-based vaccine protects against infection with multi-drug resistant and panresistant *A. baumannii* in a mouse model of disseminated sepsis. Passive immunization with anti-OMP antibodies provides rapid protective immunity against *A. baumannii* infection.

O148 Development of C5a peptidase and CspA based recombinant polypeptides as vaccine components against group B streptococci

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Objectives: Group B streptococci (GBS) is a major cause of pneumonia, sepsis, and meningitis in newborns and morbidity of immunocompromised adults. It is known that C5a peptidase and CspA allow GBS to evade phagocytosis by disrupting the C5a and several CXC chemokines of the host. The aim of the current study was to construct and investigate C5a-ase and CspA based recombinant polypeptides with predominantly α helix structure and evaluate their immunogenicity.
Methods: Collection of GBS strains consisting of 75 GBS clinical isolates from Russia, Sweden, China, USA and 10 reference strains was analyzed. PCR generated DNA fragments from cspA and scpB genes were cloned employing pQE expression vectors (Qiagen, USA). Immunological assays: 1. Subcutaneous immunization of mice and rabbits. 2. Opsonophagocytosis of GBS preliminary incubated with

normal or immune sera with mouse peritoneal macrophages. 3. Protection studies on immune mice intraperitoneally infected with GBS.
Results: All DNA samples from 85 GBS strains tested by PCR and hybridization were found to carry cspA gene. Two portions of scpB and two portions of cspA (pB1, pB3 and csp2, csp3) encoding for α helical regions of C5a-ase and CspA were amplified and cloned. Resultant proteins PB1, PB3 and CSP2, CSP3 (M.M. 12 kDa, 10 kDa and 42 kDa, 12 kDa, respectively) were successfully expressed in *E. coli* followed by purification with Ni sepharose. All polypeptides under study generated humoral immune response in mice and rabbits. The antisera were examined for their opsonizing ability against GBS strains of six different serotypes employing mouse peritoneal macrophages. The data demonstrated significant opsonizing effect of anti-PB1, anti-PB3, anti-CSP3 immune sera over the control. Mice preliminary immunized with the polypeptides were studied for development of GBS infection. As result obvious GBS clearance from the spleens of mice immunized with PB1, PB3 and CSP3 in comparison with control group was determined. In contrast anti-CSP2 antibodies were found to possess neither opsonizing ability nor protective activity in above mentioned experiments.
Conclusion: 1. DNA from 85 GBS strains comprised cspA gene. 2. PB1, PB3 and CSP3 with high α helix structure displayed the immunogenic and protective properties that allowed proposing them as vaccine components against GBS.

O149 Novel combined multiple subunit vaccine protects against extraintestinal pathogenic *E. coli*

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Objectives: Extraintestinal pathogenic *E. coli* (ExPEC) are frequent causative agents of sepsis, neonatal meningitis and urinary tract infections. They lead to significant mortality and morbidity in humans as well as live-stock and are a big burden for healthcare providers. With fast rising antibiotic resistance rates among clinical ExPEC isolates preventive vaccination against these pathogenic *E. coli* is greatly desired.
Methods: Based on genome analysis data and *in vivo* transcription studies we selectively target virulence factors expressed during the course of infection such as iron acquisition systems. Immunogenic regions as well as the unknown three dimensional structure of these proteins were analyzed in silico. MHCI and MHCII epitopes as well as proteasome cleveage sites were also taken into account.
Based on this data two synthetic modular vaccine proteins were designed each bearing eight epitope rich subfragments. For each a fully synthetic gene was constructed and optimized regarding sequence and codon bias. We used and evaluated two application routes in the mouse model. First we used purified vaccine proteins for intranasal application. Second we used a novel *Salmonella* based bacterial antigen delivery system which is simply administered orally. As it translocates the vaccine directly into the cytoplasm of immune cells *in vivo*, T-cellular response is greatly enhanced.
Results: Specific immune responses were evaluated with IFN-g ELISpot, flow cytometry and sub-class sensitive antibody ELISA. Intranasally immunized mice demonstrated massive increases of specific serum IgG as well as vaginal fluid IgA antibodies against the recombinant vaccine protein as well as against purified full length virulence factors of ExPEC. Expansion of vaccine specific IFN-g secreting T-cells could be proven in both immunization routes. In the challenge model of peritonitis a significant reduction of bacterial load could be achieved in immunized mice, demonstrating the first successful combined T-cell vaccine against ExPEC in the mouse model.
Conclusion: Combined T-cell and antibody stimulating vaccines containing multiple epitopes are effective against ExPEC in the mouse. Further evaluation is needed to elucidate the potential for use in humans.

O150 Dendritic cell vaccination generates protective responses against influenza virus infection: a model explored for effective vaccination of immunosuppressed cancer and stem cell transplant patients

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Introduction: We have recently demonstrated the ability to generate robust, antigen-specific T-cell responses following priming of naïve, umbilical cord blood lymphocytes with recombinant influenza hemagglutinin (rHA) loaded dendritic cells (DC) (Safdar et al. Vaccine 2009). Toward the goal of validating this model *in vivo*, we have developed a murine model of DC immunotherapy utilizing a Balb/c-adapted A/New Caledonia H1N1 influenza virus. Here we demonstrate that a single injection of rHA-loaded DCs can generate high antibody titers and associated with protection against lethal influenzavirus infection.
Methods: Balb/c-adapted A/New Caledonia was generated by 4 serial passages of infected lung pools. Bone marrow stem cells derived from Balb/c donors were incubated in GM-CSF and IL-4 for 6 days to generate immature DCs. DCs were loaded with A/New Caledonia rHA protein and then matured in a cocktail of GM-CSF, IL-4, IL-1β, TNF-α, IL-6, and PGE2. Mature DCs were harvested after 24 hours and delivered at a dose of 106 DC by subcutaneous (SC) or intraperitoneal (IP) routes. Mice were bled 4 weeks after vaccination for serum HA-specific antibody (Ab)titers by ELISA. Six weeks after vaccination, mice were challenged with a 10xLD50 of Balb/c-adapted A/New Caledonia influenzavirus administered intranasally. Efficacy of the vaccine was determined by survival.
Results: Four weeks after vaccination, 3 of 3 mice injected with DCs IP had seroconverted with high titers apparent at 1:250 serum dilutions. In contrast, only 1 of 3 mice injected SC demonstrated seroconversion and only at a serum dilution of 1:25. None of the mice injected with control DCs (not loaded with rHA) demonstrated rHA-specific seroconversion. At six weeks post-vaccination, mice were challenged with a 10 x LD50 of Balb/c-adapted A/New Caledonia. On day 15 post-inoculation, the four mice with HA-specific Ab titers were all alive with no significant changes in weight, appetite, or behavior, whereas the 4 mice with no demonstrable HA-specific Ab titers had all died ($p < 0.005$).
Conclusions: The model demonstrates that robust HA-specific antibody titers may be generated by a single injection of HA-loaded DCs, and that such titers are protective against fulminant influenza infection. This validation of *in vitro* data suggests that DC immunotherapy for the prevention of influenza in immunosuppressed cancer patients might be feasible and warrants further exploration of the technique.

O151 A novel DNA vaccine against toxoplasmosis induces sporozoite specific protective immune response through nonapoptotic cells

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Objectives: *Toxoplasma gondii* is an obligate intracellular parasite infecting all warm-blooded animals, including humans and causes serious clinical presentations. There is no 100% effective drug to treat toxoplasmosis. Development of a vaccine, which can prevent the consequences of acute infection, is an attractive alternative.
Since 1990s, vaccination strategies against toxoplasmosis mainly used DNA vaccine, purified recombinant proteins showed variable protection because vaccine candidate antigens were almost always selected randomly and targeted tachyzoite form which invades the host cell abruptly and immediately embeds itself in a protective parasitophorous vacuole which keeps away the host immune response.
Currently, there is substantial evidence about *T. gondii* oocysts (contains sporozoites) as being the cause of water related outbreaks. After classification of *T. gondii* oocysts in category B bioterrorism agents

as a water safety threat, the demand for a protective vaccine against toxoplasmosis has increased.
The present study aims to generate first time a DNA vaccine containing a sporozoite specific surface antigen "SporoSAG" to block the sporozoites as they are released from the oocysts in the intestine. To increase the efficacy of the vaccine, antigen specific-CD8 response inducing anti-apoptotic Bcl-xL gene was inserted to the dual expression DNA vaccine.
Methods: During the construction of the dual expression DNA vaccine, SporoSAG was inserted after CMV promoter and anti-apoptotic protein Bcl-xL were inserted in frame with EGFP after IRES (pIRES2EGFP-SporoSAG-Bcl-xL). *In vitro* transfection of BHK-21 cells was performed to show the expression of SporoSAG and Bcl-xL. The functionality of Bcl-xL was demonstrated by Casp3 flow cytometry. Humoral and cellular immune response (CD4/CD8, IFN-γ) were analyzed from sera and spleen cells of vaccinated BALB/c mice by western blot and flow cytometry using purified recombinant SporoSAG protein (Figure 1).
Results and Conclusion: Western blot and fluorescence microscopy analyses of pIRES2EGFP-SporoSAG-Bcl-xL transfected BHK-21 cells showed SporoSAG and Bcl-xL expression. Casp3 analyses rationalized Bcl-xL expression that impedes apoptotic cell death. Analysis of sera obtained from vaccinated mice showed anti-SporoSAG antibody response compared to controls. Cellular immune response analyses showed increased CD8 and IFN-γ response compared to controls indicative of protection against toxoplasmosis.

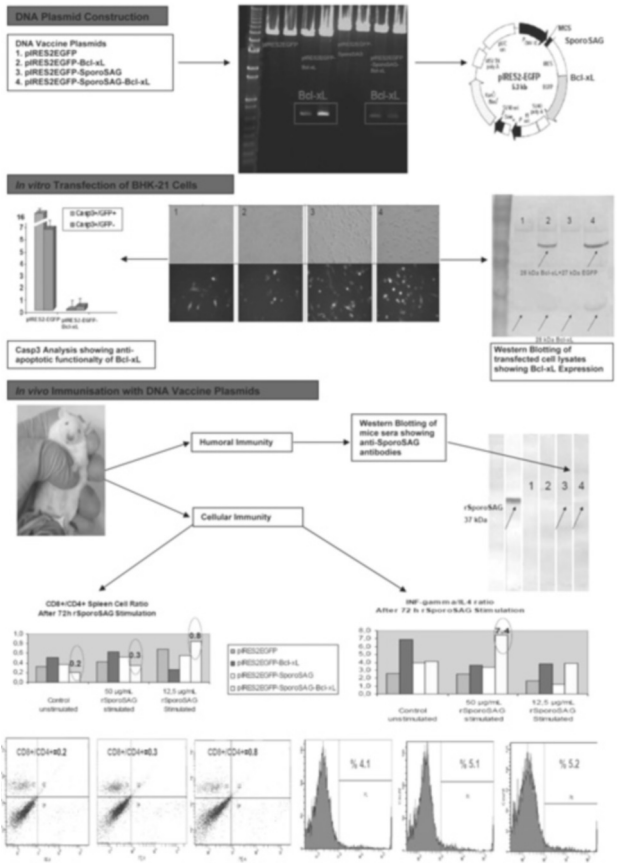

Figure 1.

O152 Assessment of a new DNA vaccine candidate against urinary tract infection

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Objective: Urinary Tract Infection (UTI) is one of the most common infectious diseases and the major agent of UTI is Uropathogenic *Escherichia coli* (UPEC). Most of the time, it has affinity to attached to the vaginal and urinary tract mucosa tissue. UPEC has intracellular propagation, when do invasion of bladder epithelial cells. Therefore, cellular immune response is so important in this case. In our research, a genetic construct for inducing of cellular immune system was designed and immune response of immunized mice was evaluated.
Materials and Methods: Chromosomal DNA extracted from *E. coli* 35218 as a positive type I pili strain and fimH gene amplified by using this template in PCR. The PCR product inserted to pBluescript cloning vector and sequenced. Then, the fimH gene sub cloned to pVax eukaryotic expression vector. The recombinant vector sequenced again. COS7 cell line transfected with a complex of pVax/fimH and ExGen 500 poly cationic polymer. Three groups of BALB/c mice immunized with recombinant DNA construct. The first group injected intramuscularly (i.m) with two doses (100 µg for every injection during two weeks) of purified pVax/fimH. The second group injected with the same amount of pVax vector and the third group injected with PBS as negative control. All mice challenged, one week following the second injection, with uropathogenic *Escherichia coli* strain 35218. Moreover, lymphocytes isolated from spleen of immunized mice and cultured for cytokines assay.
Results: The sequence of *E. coli* 35218 fimH gene in our research showed more than 97% identity to other fimH sequence reports in GenBank. Expression of fimH gene in transfected COS7 was confirmed by RT-PCR. The result of challenge showed 100 times reduction of *E. coli* colonization in bladder tissue of first group mice. Additionally, IFN-γ titer got rise in first group on compression with others groups.
Discussion: fimH gene has a little variation among type I pili positive strains but it has less variation on amino acid sequences. Hence, it was detected more than 97% identity of *E. coli* 35218 fimH sequence with others. Expression of fimH in pVax/fimH cassette was confirmed by RT-PCR. Consequently, induction of cellular immune response was showed by increasing of INF-γ titration in immunized mice. So, DNA vaccination has a potential candidate for limiting recurrent urinary tract infection.

O153 Rabies neutralizing antibody in AIDS patients after rabies post-exposure treatment with doubling the intramuscular doses of conventional regimen and aluminium-adjuvanted tetanus toxoid

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HIV-infected patients with low CD4+ T lymphocyte had a poor Nab response to conventional pre- and post-exposure rabies vaccination. Our previous reports revealed that HIV-infected patients with CD4+ counts <200 /µL did not respond well to conventional intramuscular (IM) post-exposure vaccination or multiple-site intradermal (ID) vaccination.
We conducted a prospective study of the Nab response after doubling the IM doses of WHO IM regimen (ESSEN) and aluminium-adjuvanted tetanus toxoid (TT) in AIDS persons. Fifteen AIDS patients (age range, 30–46 years) without active opportunistic infections who presented with possible or proven rabies exposure were enrolled. Four patients had severe exposure (WHO category III). All patients had a history of AIDS-related conditions such as opportunistic infections or CD4+ counts <200 /µL, Only 5 patients did not received antiretroviral therapy. All patients were given the post-exposure rabies prophylaxis. They received doubling the doses of IM regimen (2–2-2–2-2–0) with purified Vero cell rabies vaccine (doubling the IM doses of vaccine in 1–1-1–1-1–0 ESSEN-IM regimen) but one of the two rabies vaccines on day 0 was dissolved in

0.5-mL dose of aluminium-adjuvanted TT. Blood samples were obtained on days 0, 7, 14, 28, 90, 180 and 360 after vaccination for rabies Nab. CD4+ counts were determined from all patients before vaccination. Blood was drawn for determination of HIV-RNA level on days 0, 28, and 90 after vaccination. There was no serious adverse reaction among all patients treated. No death as a result of rabies during the next 1 year was reported. Seven AIDS patients with CD4+ counts <100/μL (range, 1–99 /μL) had a poor or even undetectable Nab response. Eight AIDS patients with higher CD4+ counts (>250 / μL) had good Nab responses as our previous studies. We also observed that HIV-RNA level did not increase significantly after rabies vaccination.
Conclusion: At present, cleansing the wound, vigorous wound injection with immunoglobulin, and determination of Nab response in AIDS patients who had very low CD 4 + T lymphocyte count after post-exposure rabies vaccination are the most important post-exposure measures that can be offered to AIDS and moderately or severely rabies-exposed patients.

O154 Safety of vaccination with 7-valent conjugated pneumococcal vaccine among HIV-infected adult patients

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Objectives: Patients with HIV infection have a higher incidence and recurrence rates of invasive pneumococcal diseases compared with the persons without HIV infection. An increasing number of clinical studies are reported to evaluate the immunogenicity of 7-valent conjugated pneumococcal vaccine (PCV) among elderly and transplant recipients, but the data of safety of vaccination with PCV among HIV-infected patients are sparse.
Methods: HIV-infected patients aged 18 years or greater were enrolled for vaccination with PCV to prevent invasive pneumococcal diseases in from October 2008 to October 2009. Two groups of patients were assessed: group A, recipients of 2 doses of PCV given at a 1-month interval and group B, recipients of 1 dose of PCV. Patients were further stratified to 4 groups according to CD4+ count when primary PCV was administered: group 1, CD4+ <200 cells/mL; group 2, CD4+, 200 to 349 cells/mL; group 3, CD4+, 350 to 499 cells/mL; and group 4, CD4+ ⩾500 cells/mL. All patients were given 1-week diary to record any discomfort after vaccination. All statistical analyses are performed by STATA statistical software.
Results: 407 HIV-infected patients were vaccinated with PCV during the study period: 206 patients received two doses and 201 patients one dose. After first dose vaccination, 372 (91.1%) patients who returned their one-week diary for recording side effects were assessed. Among these 372 HIV-infected patients, 124 (33.3%) patients reported discomfort after the vaccination that included injection site soreness (22.85%), pain (8.60%), redness (1.08%), swelling (1.62%), fever (1.62%), headache (1.88%), fatigue (4.30%), cough (1.34%), rash (0.53%), rhinorrhea (0.27%), sneezing (0.27%), and insomnia (0.27%). None of these patients reported severe adverse events that prompted medical attention. The only factor that was found to be associated with occurrence of side effects was high nadir CD4 counts ($P = 0.04$) before vaccination. The associated factors for injection site soreness, the most common side effect after vaccination, included high nadir CD4 counts ($P = 0.01$) before vaccination and high HIV viral load at vaccination ($P = 0.01$). None of the patients who received two doses PCV reported aggravated discomfort after second dose vaccination.
Conclusions: We conclude that vaccination with 7-valent PCV among HIV-infected patients was generally safe and high nadir CD4 counts before vaccination were associated with increased risk for injection side reactions.

O155 Nasopharyngeal carriage of *Streptococcus pneumoniae* non-vaccine serotypes among children attending day-care centres in France: 1999–2008 after the introduction of the 7-Valent Pneumococcal Conjugate vaccine

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Objectives: *S. pneumoniae* (SP) carriage is monitored among children in day care centers (DCCs) in France following implementation of 7-Valent Pneumococcal Conjugate (Pn7) vaccination (2002) and interventions promoting prudent antibiotic use (PAU).
Methods: Cross-sectional surveys were performed from January to March 1999, 2002, 2004, 2006 and 2008 on a random sample of children attending DCCs in two areas (Alpes Maritimes: area A and North: area B). For each period, antibiotic susceptibility and serotype distribution of NP SP isolates were recorded, as well as immunization status and antibiotic use. A local intervention promoting PAU was conducted in 2000 and 2003, via academic detailing visits to general practitioners and paediatricians(area A), and a national media campaign began in 2002(area A + B). Pn7 became available in 2002.
Results: SP carriage decreased in area A: 54% in 1999 to 45% in 2008 (p 0.01) and was stable in area B (46% to 42%) while the proportion of SP with decreased susceptibility to penicillin (PDSP) fell from 63% in 1999 to 42% in 2008 ($p < 10^{-3}$) in area A and remained stable 72% to 68% in area B. Antibiotic use dropped from 63% to 37% of children ($p < 10^{-3}$) in area A and from 66% to 53% in area B ($p < 10^{-3}$). Overall antibiotic susceptibility increased. Over 90% of the children had received at least one vaccine dose in 2008 in area A and 86% in area B while immunization rates were 37% and 30% respectively in 2004. A shift from Pn7 serotypes 6B, 9V, 19F, 23F in 1999 to Pn7 vacccine related 6A, 19A, 23 non-F and non-vaccine types: 15 in 2008 was observed in the two areas. Among PDSP, serotypes 6A, 19A and 15 accounted for over 50% of strains in 2008 vs. 6% in 1999 ($p < 10^{-5}$).
Conclusion: Children are now mostly colonized with vaccine-related and non-vaccine strains with improved overall antibiotic susceptibility after the introduction of pn7 vaccine; but PSDP isolates are now mainly recruited among these. Inappropriate prescriptions threaten the sustainability of vaccine-related benefits through combined vaccine- and antibiotic-driven selective pressure.

	SP (%)										PDSP (%)									
	Area A					Area B					Area A					Area B				
	1999	2002	2004	2006	2008	1999	2002	2004	2006	2008	1999	2002	2004	2006	2008	1999	2002	2004	2006	2008
23F	30.9	18.5	9.3	1.8	0.0	25.6	27.6	10.2	2.7	0.0	48.5	22.3	14.9	0.0	0.0	34.5	31.3	12.7	2.4	0.0
6B	19.8	21.4	13.1	4.7	0.6	28.2	20.7	28.4	1.4	0.0	15.8	23.2	17.2	0.0	0.0	21.4	22.2	34.5	1.2	0.0
19F	11.7	15.0	19.1	9.4	3.1	8.5	13.8	19.3	11.0	8.0	5.9	14.3	17.2	18.6	6.1	7.1	16.2	23.6	17.1	11.9
14	11.7	16.8	7.7	4.1	0.6	8.5	12.9	10.2	1.4	1.1	16.8	23.2	16.1	5.1	1.5	9.5	15.2	16.4	2.4	1.7
9V	2.5	2.9	0.0	0.0	0.0	2.6	3.4	3.4	0.0	0.0	3.0	1.8	0.0	0.0	0.0	3.6	4.0	5.5	0.0	0.0
6A	5.6	8.7	17.5	10.5	9.4	0.9	1.7	14.8	10.3	8.0	1.0	3.6	6.9	11.9	12.1	1.2	1.0	0.0	4.9	3.4
19A	3.1	6.9	9.3	10.5	15.1	8.5	6.9	1.1	28.8	23.9	2.0	8.0	14.9	20.3	34.8	11.9	8.1	0.0	51.2	35.6
23 non-F	0.6	4.0	3.3	12.3	10.1	0.0	0.9	3.4	7.5	6.8	0.0	0.9	0.0	3.4	6.1	0.0	0.0	0.0	1.2	0.0
15	1.9	1.2	4.4	9.9	20.1	0.0	3.4	2.3	12.3	22.7	3.0	0.9	4.6	13.6	16.7	0.0	0.0	0.0	8.5	27.1
Others	11.7	3.5	16.4	31.6	37.7	17.1	7.8	4.5	18.5	27.3	4.0	1.8	8.0	22.0	19.7	10.7	2.0	7.3	6.1	15.3

O156 Pili distribution among invasive pneumococci in Portugal after 7-valent conjugate vaccine

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Objective: Evaluate the distribution of the pilus islets among *Streptococcus pneumoniae* recovered from invasive infections after 7-valent vaccine availability.
Methods: Two pilus-like structures (PI-1 and PI-2) have been recently recognized in pneumococci. These have been implicated in virulence and suggested as potential vaccine targets. However these pilus-like structures are not universally distributed among pneumococcal strains. We had previously demonstrated that carrying PI-1 was a clonal property of *S. pneumoniae* and that only 27% of the invasive strains carried the rlrA islet. Furthermore, 83% of these piliated strains expressed vaccine serotypes. Similar findings were reported for the PI-2 islet which was shown to be present in 16% of a convenience sample of strains. PI-2 positive clonal complexes were associated with serotypes 1, 2, 7F, 19A,

and 19F. The introduction of the 7-valent conjugate vaccine (PCV7) resulted in changes in serotype frequency that can also affect pili prevalence. To evaluate this effect we determined the presence of PI-1 and PI-2 among a collection of 475 invasive isolates recovered from children after PCV7 introduction (2003–2008). The results were analyzed in terms of pilus islet association with antibiotic resistance, serotype, pulsed-field gel electrophoretic profile (PFGE) and multilocus sequence type.
Results: Overall, 46.7% of the strains presented one of the pilus islet. As observed in previous studies serotype distribution analysis showed a high correspondence between serotype and the presence and type of pili (Wallace coefficient, W = 0.85). This association was even higher when considering pili and PFGE cluster (W = 0.98). The rlrA islet was identified in 13% of the strains most of them expressing serotype 6B, 9V, 14, 19A and 19F. PI-2 islet was found to be present in 37% of the pneumococcal strains and was identified mainly among serotypes 1 and 7F.
Conclusion: A decrease in the presence of the rlrA islet among invasive pneumococcal strains was observed after PCV7 availability. This change is associated with the decrease of vaccine serotypes since the majority of the strains carrying PI-1 expressed vaccine serotypes. In contrast, PI-2 islet was more prevalent due to the predominance of serotypes 1 and 7F. Since most of the strains carrying pili presented serotypes that are included either in current or future conjugate vaccine formulations, their potential use in a vaccine would offer limited additional benefits.

Epidemiology and control of *C. difficile* infection

O157 Final results of the first pan-European *Clostridium difficile* infection survey

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Objectives: To survey the incidence and demographic, clinical and microbiological characteristics of *Clostridium difficile* infection (CDI) in hospitals in Europe.
Methods: We organised a network of 106 laboratories capable of isolating *C. difficile* strains in 34 European countries. In November 2008, 1–7 hospitals per country, depending on population size, tested stool samples of patients aged >2 years who were suspected of CDI, or who had developed diarrhoea after ⩾3 days of hospital admission. CDI was defined by a positive enzyme immunoassay for *C. difficile* toxin A and/or B, a positive cytotoxicity test, or positive culture of a toxigenic strain. Each hospital collected detailed clinical data of the first 10 CDI cases, and sent stool isolates of *C. difficile* for PCR-ribotyping and characterization of toxin A, toxin B and binary toxin genes. After 3 months, follow-up clinical data were collected.
Results: CDI incidence varied across hospitals (mean: 5.5 per 10,000 patient-days per hospital; range: 0 to 36.3). Detailed information was obtained on 509 patients and 395 isolates of these patients were available for characterization. Sixty-two different PCR ribotypes were found, among which 014 (15%), 001 (10%) and 078 (8%) were most prevalent. The prevalence of PCR ribotype 027 was 5%. Most patients had the previously identified risk profile of an elderly patient with co-morbidity and recent antibiotic use. At follow-up, 22% of patients had died and in 40% of these deaths CDI played a role. In multivariate analysis, age ⩾65 yrs, use of macrolides and infection by PCR ribotypes 015, 018 and 056 were significantly associated with death to which CDI contributed. Ceftazidime use and having recurrent CDI at inclusion were significantly associated with recurrences during follow-up.
Conclusion: In this pan-European, hospital-based study, the incidence of healthcare-associated CDI varied widely between hospitals. The overall and attributable mortality were high. Dominant PCR ribotypes were 014, 001 and 078, whereas 015, 018 and 056 were associated with a complicated disease course. These data emphasise the importance of multi-country surveillance and indeed that PCR ribotypes other than 027 are prevalent causes of CDI in Europe.

O158 Epidemiological and microbiological characteristics of *Clostridium difficile* infections, France, 2009: a national, multicentre, prospective survey

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Background: Following the emergence in 2006 of the PCR-ribotype 027 epidemic clone, surveillance of *Clostridium difficile* infections (CDI) in France was reinforced through the mandatory notification of severe cases or clusters in healthcare facilities (HCF). To complete this strategy, the national public health surveillance institute (InVS) and the *C. difficile* national reference centre (NRC) launched a multicenter, national, prospective survey in 2009.
Objectives: To assess CDI incidence in HCF and the geographical distribution and characteristics of strains responsible for CDI.
Methods: From March to August 2009, voluntary HCF reported through a web-based questionnaire the total number of new CDI patients by origin and severity, admissions and patient-days (pd). ECDC case definitions were used and data were stratified by type of wards: acute-care (AC) vs. rehabilitation/long-term care (RLTC). HCF were asked to send to the NRC strains isolated in March from patients diagnosed with a CDI for characterisation and typing.
Results: 137 HCF participated in the epidemiological component of the survey. On 1st November 2009, a complete dataset was sent by 85 (73%) of 117 HCF with AC wards and 72 (69%) of 105 HCF with RLTC wards. In AC, 1,045 CDI-patients were reported; CDI incidence was 2.18 cases per 10,000 pd (1.05 case per 1,000 admissions). By origin, 686 (66%) cases were healthcare-associated (HA), 598 (87%) of which were acquired in the reporting HCF; 294 (28%) community-associated (CA); 65 (6%) of unknown origin (UO). Among 734 CDI-patients diagnosed in HCF actively following them up for a month, 100 (14%) were severe cases and 25 (3%) died from their CDI. In RLTC, 251 CDI-patients were reported; CDI incidence was 1.31 cases per 10,000 pd. By origin, 231 (92%) cases were HA, 204 (88%) of which were acquired in the reporting HCF; 8 (3%) CA; 12 (5%) of UO. Among 186 CDI-patients diagnosed in HCF actively following them up for a month, 4 (2%) were severe cases and died from their CDI. Last, 237 *C. difficile* strains were sent by 54 HCF to the NRC; their characterisation and typing are underway.
Conclusions: Preliminary results from this survey confirm that CDI incidence in France is much lower than reported in other European countries, and suggest that CDI are adequately controlled in French HCF. However, the high proportion of CA cases reported in AC wards suggests the need for further studies. Final results from this survey will be presented in April.

O159 Prevalence of *Clostridium difficile* in retail meat products in north-eastern Italy

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Recent studies conducted in Canada, United States and Sweden pointed out that *Clostridium difficile* spores are present in retail ground meat thus suggesting that meat could act as food source of *Clostridium difficile* for human infections.
Objectives: The aim of this pilot study was to estimate the occurrence of *Clostridium difficile* in minced beef and pork at retail in North-Eastern Italy.
Methods: The cross-sectional study was performed in February-March, 2008, in 150 retail outlets randomly sampled in 3 provinces of North-Eastern Italy. Among those 3 provinces retailer's sample size was proportional to resident population. In each sampled retailer one laboratory sample of minced beef and one of pork was randomly collected. Each sample was microbiologically processed using the *Clostridium difficile* selective medium Cefoxitine Cycloserine Fructose broth (added with Taurocolate). After 10 days of incubation broth cultures were plated on blood agar base, horse blood red cells added

(5% v/v), and tested by PCR for detection of *Clostridium difficile* DNA according to Penders (2005). The presence of toxins A and B in the broth culture was evaluated by Techlab C.diff quik chek complete kit (Techlab). A positive control (meat experimentally infected with *Clostridium difficile* spores) was carried out simultaneously with each batch of the experimental samples.

Results: In total 300 (150 pork and 150 beef) samples were analyzed. All samples were negative for *Clostridium difficile* both at the microbiological and PCR analysis. Search for Toxin A and B gave negative result as well.

Conclusion: Although public health relevance of *Clostridium difficile* contaminated food is yet to be clarified, these preliminary results prompt to consider that ground beef and pork do not represent a risk factor for human infection in North-East of Italy. Our findings are supported by those of Von Abercron (2009), who found a low prevalence (2%) of *Clostridium difficile* contaminated ground meet in Sweden. Differently Rodriguez-Palacios (2007) and Weese (2009) in Canada, and Songer (2007) in United States reported 12–20% and 42%, respectively, prevalence of *Clostridium difficile* contaminated ground meat. These apparently conflicting results may be due to different prevalence of *Clostridium difficile* at fatted calf and swine primary production or to different hygiene both at slaughterhouse and at retail outlets. The hypothesis should be further investigated.

O160 A review of the epidemiology, risk factors and strain characteristics of *Clostridium difficile* among hospitalized patients: a pilot nested case–control study

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Introduction: The study aimed to determine the outcomes, mortality and morbidity from *Clostridium difficile* infections (CDI) in the North West of England, and to identify possible differences in risk factors between ribotypes.

Methods: Multicentre case control study at three large hospitals in NW England. Cases defined as hospitalized patients with positive *Clostridium difficile* toxin in diarrhoeal stool samples. Controls randomly selected from matched patients with negative stool samples, at a ratio of 1:1, frequency matched by 10 year age band. Data was abstracted at case note review. Stool samples from cases cultured and typed at regional reference laboratory.

Results: Between Sept 9, 2007 and Sept 30, 2008, data on 296 cases and 296 controls collected and analysed. Average age for controls and cases was 77 years. 40.2%(119/296) of controls and 48.3%(143/296) of cases were male. 48 (16%) controls and 97 (33%) cases died during the study period. Of the samples typed, 27.7% were 001, 26.6% were 001 and 20.9% were 027.

In the overall logistic regression model, length of stay, transfer from another NHS hospital, number of different antibiotics, number of days of quinolone antibiotics, degree of comorbidity and number of days of nitroimidazole antibiotics were identified as risk factors (see figure). Reduction in odds of CDI with H2-blocker therapy was an interesting finding.

On subgroup analysis the three main strains were all associated with length of hospital stay and transfer from another NHS hospital. All were associated with antibiotic use, especially quinolones. Only ribotype 001 was associated with cephalosporin use. PPI therapy was only a risk factor for ribotype 106.

The 014/020, 078 and 027 ribotypes had the highest observed rates of mortality and morbidity, but there were no significant difference in mortality or morbidity detected between different ribotype.

Discussion: This study did not find any difference in mortality or morbidity between different strains of *Clostridium difficile*, but did find some differences in risk factors between ribotypes. An unexpected association of H2-blocker therapy with a reduction in odds of CDAD was observed.

This study has some limitations. Diagnosis of *Clostridium difficile* associated disease was based on toxin detection, which may not be sensitive or specific. Details to be presented.

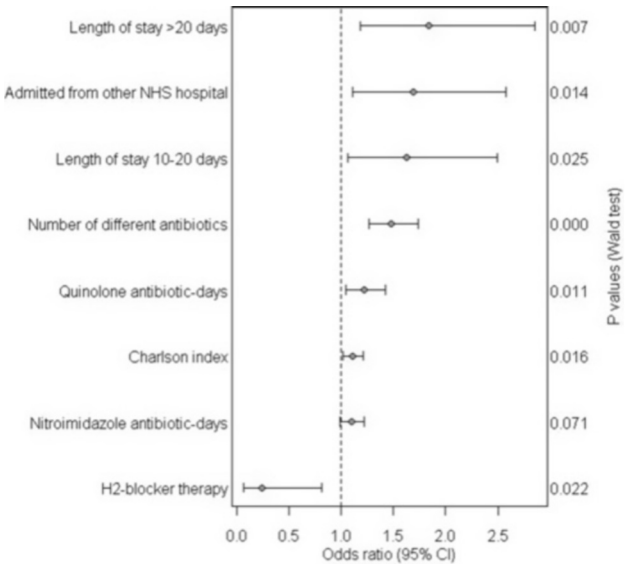

Figure. Multiple logistic regression model for all ribotypes.

O161 Stronger correlation between antibiotic consumption and incidence of *Clostridium difficile* determined by cultures instead of faecal toxin only

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Objectives: The diagnosis of *Clostridium difficile (C. difficile)* associated diarrhoea is usually based on the detection of faecal toxin A/B, rather than on stool cultures. Therefore, studies on the correlation between the incidence of *C. difficile* and antibiotic consumption were limited to toxic strains in the past. The aim of this ecological study was to analyse the association between unit-specific antibiotic consumption and the incidence of toxigenic and non-toxigenic *C. difficile* in hospitalised patients.

Methods: Unit specific antibiotic consumption data and the incidence of *C. difficile* in 19 units over 5 years were analysed at the University Hospital of Basel, Switzerland. Stool samples were tested for toxin A/B and simultaneously cultured. In a second step, positive cultures were tested for toxin production. In order to analyse specific antibiotics, unit specific length of stay and antibiotics showing a significant association in univariate analysis were entered in a multiple linear regression model.

Results: Over 5 years, a total of 165 first isolates of toxin producing *C. difficile* and 413 first isolates of *C. difficile* were detected. The incidence of *C. difficile* overall and of toxin producing *C. difficile* was 0.39 and 0.15 per 1000 patient days, respectively.

The correlation (figure) is highly significant in both analyses ($p < 0.001$), but a higher correlation results when all *C. difficile* strains are included in the model ($R = 0.80$ vs. $R = 0.63$). In a multivariate analysis explaining more than 80% of the variance of the incidence of *C. difficile* (R2=0.82), only piperacillin/tazobactam, trimethoprim/sulfamethoxazole, and the aminoglycosides were found to be statistical significant risk factors.

Conclusion: We show for the first time, that the correlation between antibiotic consumption and the *C. difficile* incidence rates significantly improves, if detection is not limited to toxin producing strains. Overall antibiotic pressure as a risk factor was previously underestimated due to restriction to the toxin producing strains. Our results underline the importance of the overall pressure of antibiotics that explains the majority of the variance rather than the importance of a single group of antimicrobial agents such as quinolones. Therefore, interventions to

reduce the overall antibiotic consumption might be more successful in controlling *C. difficile* incidence than interventions focusing on certain groups of antibiotics.

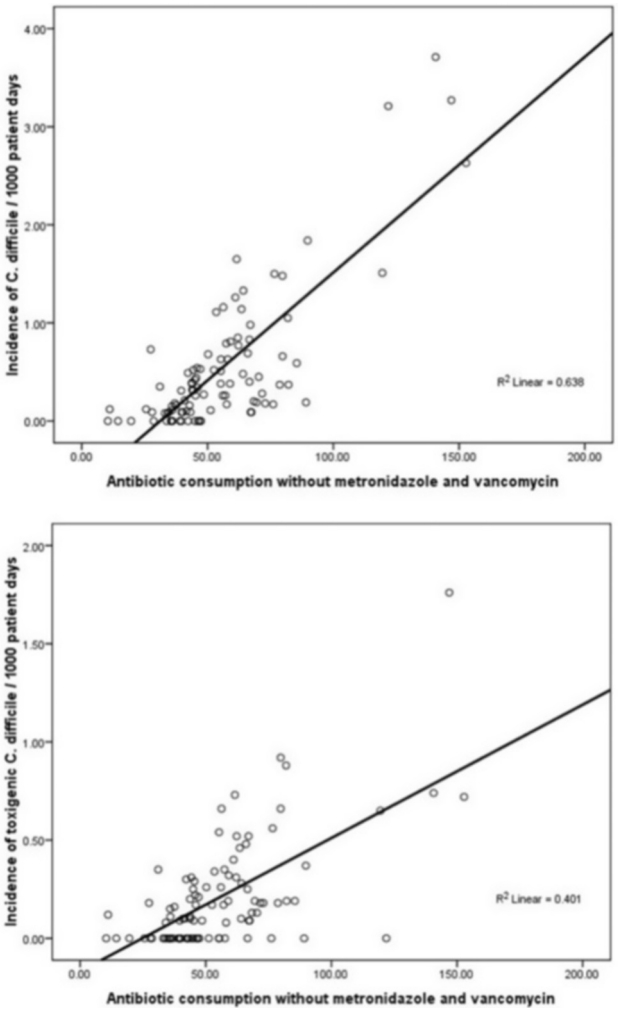

Figure. Correlation between unit-specific antibiotic consumption and incidence of *C. difficile*.

O162 Risk factors associated with *Clostridium difficile* infection in hospitalized patients with hospital-onset healthcare facility-associated *Clostridium difficile* infection

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Objective: To assess risk factors associated with *Clostridium difficile* infection (CDI) in hospitalised patients with hospital-onset healthcare facility-associated (HO-HCFA) CDI.

Methods: This retrospective analysis utilised data from April 1, 2005 to December 31, 2008 within Cerner's Health Facts® database. Qualifying cases were adults with an inpatient encounter with a primary or secondary diagnosis of pseudomembranous colitis (ICD-9-CM code: 008.45) AND a positive *C. difficile* toxin test AND the first positive *C. difficile* test (index event) was ordered more than 72 hours after admission. Non-CDI controls were hospitalised for at least 72 hours with no diagnosis of pseudomembranous colitis AND no *C. difficile* culture or toxin assays during the study period. Stepwise logistic regression with bootstrapping and a Bayesian Information Criterion analysis were used to identify variables with the strongest association with HO-HCFA CDI. Logistic regression was used to quantify the variables' effect on HO-HCFA CDI. The area under the receiver operating characteristic (AUROC) curve and Hosmer-Lemeshow (HL) statistic were used to assess the model's overall fit.

Results: Among 34,261 inpatients, 1,454 met the study definition of HO-HCFA CDI. A final model of 26 variables (AUROC = 0.89, HL statistic = 10.82, P = 0.21) is reported in the table. Factors most associated with increasing the odds of CDI include evidence of impaired immune function (odds ratio [OR] = 4.77; 95% CI, 3.73–6.10), an unknown infection source (OR = 4.35; 95% CI, 2.92–6.46), and being admitted from a skilled nursing facility (OR = 3.47; 95% CI, 2.39–4.92). The antibiotic tigecycline was associated with lower risk of CDI (OR = 0.21; 95% CI: 0.06–0.76).

Conclusions: Impaired immune function, a high comorbidity burden, advanced age, and the presence of other infections were seen as risk factors for HO-HCFA CDI. Antibiotic therapy has been known to play a central role in the pathogenesis of CDI but the observation of tigecycline exposure's protective effect is unexpected. Additional research is needed to better understand the differential risks and benefits of various broad spectrum antibiotics among patients at risk for HO-HCFA CDI.

Table. Significant risk factors associated with *Clostridium difficile* infection

Variable	Odds ratio	95% CI	*P* value
Evidence of impaired immune function	4.767	3.725–6.101	<0.001
Admitted from skilled nursing facility (referent = ER admission)	3.470	2.387–4.922	<0.001
Admitted from unknown location (referent = ER admission)	2.799	1.540–5.088	0.001
Gram-positive infection	1.801	1.531–2.118	<0.001
Age (per 10 year increase)	1.151	1.054–1.258	0.002
Charlson Comorbidity Index (per unit)	1.118	1.086–1.152	<0.001
Southern hospital location (referent = Northeast)	0.404	0.208–0.783	0.007
Hospital beds: 300 to 499 (referent = 500 or more beds)	0.357	0.160–0.795	0.012
Unknown race (referent = White)	0.118	0.032–0.438	0.003
Chronic conditions on or 12 months prior to hospital admission			
Acute renal failure	2.663	2.246–3.158	<0.001
Cerebrovascular disease other than intracranial/intracerebral hemorrhage	2.391	1.624–3.519	<0.001
Heart failure	1.746	1.436–2.123	<0.001
Cardiac dysrhythmias	1.681	1.421–2.002	<0.001
Infection source			
Other or unknown	4.347	2.924–6.464	<0.001
Respiratory	2.059	1.670–2.538	<0.001
Skin infection	2.052	1.766–2.384	<0.001
Device/implant/graft	1.935	1.444–2.593	<0.001
Gastrointestinal other than CDI	1.649	1.346–2.020	<0.001
Genitourinary	1.472	1.225–1.768	<0.001
Total number of systemic antibiotic (by drug class) received before index event (referent = 0)			
2	1.541	1.250–1.900	<0.001
⩾3	1.821	1.418–2.338	<0.001
Use of medications before index event			
Miscellaneous antibiotics	2.587	1.992–3.361	<0.001
Carbapenems	2.090	1.375–3.177	0.001
Third/fourth generation cephalosporins	1.640	1.449–1.857	<0.001
Proton pump inhibitors	1.608	1.342–1.927	<0.001
Tigecycline	0.212	0.059–0.758	0.010

ER: emergency room; CDI: *Clostridium difficile* infection.

O163 Higher mortality of *Clostridium difficile* possessing the genes for the binary toxin in addition to the genes for toxin A and toxin B

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Objectives: *Clostridium difficile* PCR ribotype 027 may cause severe disease with high mortality. Only a few studies have evaluated whether the outcome of infection is associated with the toxin profile and PCR ribotypes of the infecting strain. *Clostridium difficile* (CD) isolates are referred for typing to the Danish National Reference Laboratory according to the criteria for mandatory submission as part of the Danish CD surveillance programme. Our aim was to assess the 30-day mortality of infections caused by [1] unselected isolates not referred for typing and the following groups of submitted and typed CD: [2] CD possessing the genes for Toxin A, Toxin B and the binary toxin, PCR ribotype 027 (CD027); [3] CD possessing the genes for Toxin A, Toxin B and the binary toxin, PCR ribotype non-027 (CD non-027); [4] CD possessing only the genes for Toxin A and Toxin B (CD A and B).

Methods: Laboratory based registries and the Civil Registry with demographic information on all people resident in Denmark including the date of death were used to design a cohort study. Each patient was

included only once in any of the four groups and only with the first episode of infection. Poisson regression was used to estimate the relative risk of death within 30 days after infection.
Results: A total of 54/193 CD 027 (28%), 20/72 CD non-027 (28%), 36/212 CD A and B (17%), and 247/1822 unselected (14%) patients died within 30 days after infection (log rank test, $p < 0.001$). The relative risk of death in patients with the binary toxin (CD027 and CD non-027) was 1.8 (95% CI 1.2–2.7) compared to patients without the binary toxin (CD A and B). In a multivariate analysis after adjustment for age, sex and geographical region, the relative risk was 1.6 (95% CI 1.0–2.4).
Conclusion: An identical mortality was seen for patients with CD strains possessing the binary toxin – irrespective of the PCR ribotype (CD027 or CD non-027). The mortality for patients with CD A and B strains was significantly lower than among patients with CD027 and CD non-027 but higher than among unselected patients. The results suggest that the binary toxin either is a marker for more virulent CD strains or contributes directly to the virulence of these strains.
Furthermore, the data confirms that criteria used in the Danish CD surveillance programme ensure molecular characterisation (genotypic toxin profiling and PCR ribotyping) of the most virulent strains enabling detection of emerging hyper virulent strains.

O164 Impact of different antibiotic treatment regimes for respiratory tract infections on emergence of *Clostridium difficile*

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Background: It has been suggested that treatment of lower respiratory tract infections (LRTI) with broad spectrum antibiotics and newer fluoroquinolones in particular, contributes to selection for *Clostridium difficile* (C. diff). We studied the prevalence of C. diff carriage, selection for C. diff and development of C. diff infection (CDI) in patients (pts) hospitalized and treated for LRTI and compared the different treatment regimes.
Methods: Pts receiving antibiotic treatment for LRTI in one university medical center and an affiliated teaching hospital were prospectively followed for 30 days with careful monitoring of the development of diarrhoea. Of all patients, stool samples collected on admission, day 5, day 3 after discontinuation of antibiotics and day 30 were cultured for C. diff using selective plates. Cultured isolates were further characterized for toxin production and typed by PCR ribotyping. CDI was defined as the presence of diarrhoea and a positive faeces toxin test.
Results: Of 107 pts included, 45 (41.1%) pts were treated with moxifloxacin, 47 (43.9%) with β-lactam monotherapy and 15 (14.0%) with β-lactam/macrolide combination therapy. The endemic rate of CDI in the participating hospitals is 0.6/1000 pts a year. In total, during the study period, C. diff was cultured in 18.7% (n = 20) of pts. 6 (30%) isolates were toxin positive. On admission, baseline prevalence of C. diff carriage was 10.4% (n = 10). Of those, one patient had mild CDI and persistent positive cultures for C. diff during the study period. The overall acquisition rate of C. diff carriage after antibiotic treatment for LRTI in our population was 10.3% (n = 11). Acquisition rates of C. diff carriership were 11.1% (n = 5) in moxifloxacin, 10.6% (n = 5) in β-lactam and 6.7% (n = 1) in β-lactam/macrolide treated pts (p = NS). Tests for faeces toxin production were negative in all pts who acquired C. diff. No acquired CDI or relapse of previous CDI occurred during the study period. Risk factors for C. diff carriership at any time were intravenous antibiotic treatment >7 days (OR 3.89, 95% CI 1.3–11.8) and hospitalization the past 3 months (OR 4.08, 95% CI 1.4–11.9).
Conclusions: In a setting with a low endemic rate, acquisition rates for C. diff during antibiotic treatment for LRTI were 10% and did not lead to CDI. Moxifloxacin was not associated with increased acquisition rates for C. diff as compared to other antibiotics classes prescribed for LRTI.

O165 Successful control of an outbreak of diarrhoea in a vascular surgery unit caused by a high-level clindamycin-resistant *Clostridium difficile* PCR ribotype 106

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Objective: Description of an outbreak of *Clostridium difficile* infection (CDI) affecting nine patients in a vascular surgery ward over a 6 week period in 2009 caused by a high level clindamycin resistant PCR ribotype 106 (clindamycin MIC > 256 mg/L).
Methods: *C. difficile* outbreak was suspected on the basis of three cases within 30 days and departure from the normal pattern. A case of CDI was defined as a patient with diarrhoea, positive for *C. difficile* toxin and negative for other enteric pathogens. Cultures were sent to Scottish Reference Laboratory (SSSCDRL) for PCR ribotyping, antibiotic susceptibility testing and PCR detection of ermB.
Results: The ward has 24 beds (6 side rooms). The outbreak involved 9 patients: two diagnosed post discharge. The mean age of patients was 73 years (range 38–90 years). Eight patients were male. All had significant underlying medical disorders and received clindamycin and ciprofloxacin. This was in accordance with the approved and long standing antibiotic policy of the unit. None of the cases had severe complications of *C. difficile*. All nine cases were typed as PCR ribotype 106 and they showed high level resistance to clindamycin suggesting cross transmission on the ward. Five of these isolates were tested by PCR for the presence of the ermB gene and no amplification was detected. This strain has rarely been isolated outwith this ward. The outbreak was controlled successfully by closure of the ward with terminal cleaning using a hypochlorite solution, reinforcement of infection control precautions especially hand washing, stopping the use of ciprofloxacin and clindamycin and the introduction of a new antibiotic policy. There have been no new cases of *C. difficile* for over 120 days since the last outbreak case was diagnosed.
Conclusion: This report demonstrates that outbreaks of CDI can be caused by *C. difficile* PCR ribotypes other than 027. This outbreak was caused by an unusual strain that displayed high level clindamycin resistance, not mediated by ermB and belonged to PCR ribotype 106. The outbreak was most likely associated with the use of clindamycin and cross infection with spores in this environment. This report highlights that implementation of strict infection control precautions, antimicrobial stewardship and enhanced environmental cleaning are key components in managing such an outbreak successfully. Interestingly the number of MRSA acquisitions also dropped dramatically after these interventions.

O166 The value of real-time monitoring of the comprehensive *Clostridium difficile* infection containment programme and impact of rise in clindamycin usage (daily defined doses)

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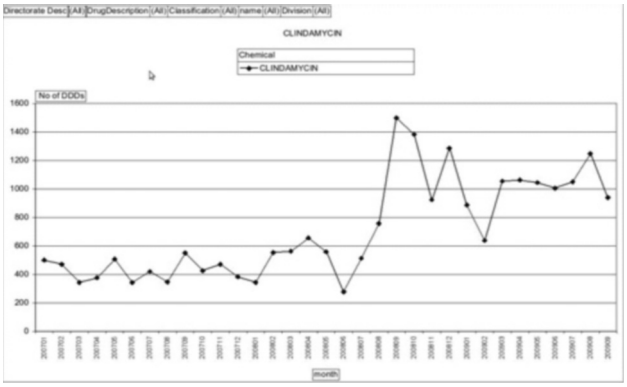
Background: Clindamycin (CD) has been widely reported in literature to be associated with high risk for *C. difficile* infection (CDI). High-dose CD (daily defined doses = DDD data) use for variety of clinical indications has steadily increased in last 3-yrs in Blackpool Victoria Hospital (large district hospital). A *Clostridium difficile* infection containment programme (CCDCP) was introduced with an aim to reduce avoidable CDI, associated morbidity, mortality & costs and increase quality of care and patient safety. The rates of CDI in trust have declined since introduction of CCDCP. Real time monitoring through 2 multidisciplinary audits (Feb-Apr07 & Sep-Dec08), multicentre regional health protection agency led nested case–control (296 each) study on CDI and rootcause analysis of every case (Jan–Oct 2009). Findings(incl. data on use of CD) from these have been used to inform and fine tune the program.
Methods: Data (2007–09) on rates of CDI; CD usage (in DDD); key results of the three studies (as above). To be presented – CCDCP package of interventions (eg. Strengthening infection control team,

quinolones/3rd gen.cephalosporin restrictions; root cause analysis (RCA) of every case; deep clean programme; PEAT audit; hand hygiene & ban-the-bug campaign; revised antibiotic formulary; 5-day stop empiric antibiotic policy;HCAI ward rounds; high-cost antibiotic rounds; revised CDI guidance; integrated care pathway, etc).
Results: post-48 h CDI rates have reduced by 58% (0.47/1000 beddays Apr-Oct09 & estimated v/s 1.13/1000 beddays 2007–08). Monthly CD usage between 300–1500 DDD were reported. Nil (63 analysed cases) & 1(59 analysed) case of CDI with previous exposure to CD within 30-days were recorded in the two audits while the regional case control study observed a 2%(cases) & 0.7%(controls) [p value 0.286 using Fisher's exact test] for all ribotypes. RCA(Jan–Oct09) revealed 55% avoidable/probab avoidable CDI; non-compliance to formulary and inappropriate, repeated or prolonged courses of antibiotics the predominant association.
Conclusions: 80% of patients in the two audits were over 65yrs of age. CCDCP includes a variety of interventions as a package. Post-48 h CDI rates are declining. The results from the two whole health economy CDIaudits, RCA and case control study were used to inform/modify the program. New CDI action plan has been drafted to address current challenges. High dose (600 mg q6 h) CD use has steadily increased over last 3-years while the three studies and RCA reveal minimal association. Antibiotic resistance to the ribotypes awaited.



Azithromycin – new horizons (Symposium supported by Teva)

S171 Azithromycin in the treatment of chronic bacterial prostatitis

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Prostatitis is an inflammatory condition of the prostate that presents with urethral symptoms, prostatic symptoms and sexual dysfunction. It is diagnosed by clinical symptoms and signs, the microscopy of expressed prostatic secretion (EPS) and culture of EPS and segmented urine samples. Prostatitis is chronic when symptoms have been present for at least 3 months. As a part of several scientific research projects we have been prospectively investigating prostatitis syndrome at the Outpatient Department of Urogenital Infections and Sexually Transmitted Diseases, Dr Fran Mihaljevic University Hospital for Infectious Diseases, Zagreb, Croatia, since March 1, 1999 and is still ongoing. We examined more than 3450 patients over 18 years of age with symptoms of chronic prostatitis and no evidence of lower genitourinary tract abnormalities.
Azithromycin fulfills the criteria for the treatment of chronic prostatitis and sexually transmitted infections – high efficacy against the most common bacterial agents of urogenital and sexually transmitted infections, acceptable toxicity and tolerability, simple oral use, excellent penetration through chronic inflammatory changed prostate capsule, achieves high concentrations in prostatic tissue and slowly eliminates for 4–8 weeks, has anti inflammatory and immunomodulatory effect, and is active against bacterial biofilm.
At total of 307 patients with chronic prostatitis caused by *Chlamydia trachomatis* were treated with azithromycin in a total dose of 4.0, 4.5 or 6.0 g for 3–4 weeks. Eradication of *Chlamydia trachomatis* was achieved in about 80%, with 70% of patients clinically cured. There were no statistically significant differences between these three dosage regimens. A total of 82 patients with chronic prostatits caused by *Ureaplasma urealyticum* were treated with azithromycin in a total dose of 4.5 g for 3 weeks. The eradication of *Ureaplasma urealyticum* was 85% and clinical cure 76%.
Conclusion: azithromycin is an effective and safe antimicrobial drug for the treatment of chronic prostatitis caused by *Chlamydia trachomatis* or *Ureaplasma urealyticum*. It is recommended in a total dose of 4.5 g po. during 3 weeks, administered for 3 days weekly in a dose 1x500 mg po. per day.
The results of recently published clinical studies on the treatment of chronic bacterial prostatitis with combination therapy with ciprofloxacin and azithromycin are impressive. We also have a limited, but positive experience.

Azithromycin – new horizons (Symposium supported by Teva)

S172 The role of azithromycin in the treatment of acute infectious gastroenterocolitis

D. Vukelic (Zagreb, HR)

Acute infectious gastroenterocolitis is a common disease worldwide. In most cases, gastroenterocolitis does not require antimicrobial treatment as it is considered a self-limiting and mild disease of short duration. However, antibiotic therapy is indicated in most severe cases. HIV-positive or immunocompromised patients should also receive antimicrobial treatment. In such cases, antibiotics could decrease the duration of the disease, enable a faster recovery and shorten the carrier state. Azithromycin proved effective in the treatment of acute infectious gastroenterocolitis. Two studies involving US military personnel in Thailand indicated that a single azithromycin dose (1 g) or a 3-day regimen (500 mg/day) were comparable or superior to ciprofloxacin or levofloxacin in empirical treatment of acute diarrhea. Efficacy of azithromycin in the treatment of traveler's diarrhea in infants and children has also been suggested.
Campylobacter enterocolitis is the most frequent form of acute bacterial diarrhea affecting humans, particularly children and young adults.
We evaluated the efficacy and tolerability of a single oral azithromycin for the treatment of *Campylobacter enterocolitis* in children $\leqslant$12 years of age, administered early in the course of the disease compared to a standard 5-day erythromycin regimen or no antibiotic. Our study suggests that a single azithromycin 30 mg/kg administered early in the course of *Campylobacter enterocolitis* in children $\leqslant$12 years of age effectively eradicates the pathogen and accelerates clinical cure attainment and that it is clinically superior to an early commenced 5-day erythromycin regimen.

S173 Current and future issues in resistance of respiratory pathogens: is the horizon still bright?

R. Kozlov (Smolensk, RU)

Respiratory tract infections (RTIs) continue to be the major causes of morbidity and mortality worldwide. Lower respiratory tract infections (LRTIs), including CAP, were ranked third in a list of the 30 leading causes of death worldwide in 1990. Out of all pathogens, *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and "atypical" pathogens still remain to be the most prevalent ones, independently on the country. Traditionally, β-lactamase, modern macrolides and respiratory fluoroqinolones (for adults only) are drugs of choice for the therapy of RTIs. But antimicrobial resistance among these pathogens continues to be the major challenge for the physicians in all countries. A total of 2,449 *S. pneumoniae* from 23 cities of Central, North-Western, Southern, Privolgsk, Ural, Siberian and Far-Eastern regions of Russia were studied from 1999 to 2009. β-lactams

retained comparatively high *in vitro* activity against *S. pneumoniae*: non-susceptibility to penicillin, amoxicillin/clavulanate and ceftriaxone/cefotaxime was 9.7%, 0%, 1.8% in 1999–2003; 8.1%, 0.3%, 2% in 2004–2005 and 14.5%, 0.6%, 4.4% in 2007–2009, respectively. Resistance to macrolides (including azithromycin) was low and varied from 2.0% to 8.2% in 1999–2003, 4.3% to 6.6% in 2004–2005 and 7.9–10% in 2007–2009 with no statistically significant changes over indicated period. Susceptibility to chloramphenicol varied from 89.4% to 94.1%. Resistance to tetracycline was high during 1999–2009 (23.7–29.6%). Non-susceptibility to co-trimoxazole increased from 31.7% in 1999–2003 to 41.3% in 2007–2009 ($p < 0.0001$). Levofloxacin and moxifloxacin retained high *in vitro* activity against *S. pneumoniae*. Proportion of multiresistant *S. pneumoniae* did not exceed 12% in studied period. Ampicillin resistance in *Haemophilus influenzae* was 5.4% with no resistance to amoxicillin/clavulanate. Resistance to azithromycin wass found to be 1.5%. *Moraxella catarrhalis* seems to be of low epidemiological importance in Russia, being isolated only in rare cases from patients with otitis media and acute exacerbation of chronic bronchitis with virtually all strains being β-lactamase producers and retaining susceptibility to β-lactam/β-lactamase inhibitors. In conclusion, all above three classes of antimicrobials retain their place in treatment of RTIs.

S174 Clinical effectiveness of azithromycin in an era of multidrug resistance: results of a prospective international, multicentre observational study (SUPoRTI)

B. Baršic (Zagreb, HR)

Azithromycin after twenty years of extensive use is still one of the most prescribed antibiotics in the treatment of bacterial, respiratory tract infections. Multidrug resistance (MDR) is reported worldwide, but clinical consequences of MDR still remain controversial. SuPoRTI study was designed to evaluate efficacy of a 3-day azithromycin therapy in the treatment of respiratory tract infections in adults and children and to compare results with early clinical trials. SuPoRTI study is a multicenter, prospective non-comparative phase IV study, which included 550 patients with bacterial upper and lower respiratory tract infections. Three countries and 26 centers participated in the study. Only outpatients were included in the study. The trial included patients with acute pharyngitis, sinusitis and otitis media as well as patients with acute exacerbation of chronic bronchitis and mild to moderate community acquired pneumonia. Azithromycin was administered orally. Clinical effectiveness was assessed after the third or fourth visit and dynamic of clinical scores was assessed throughout the study. We present the results of effectiveness and safety of azithromycine obtained in this study.

Non-typeable *Haemophilus influenzae*, the "unrevealed pathogen" (Symposium supported by GlaxoSmithKline Biologicals)

S178 The role and pathogenicity of non-typeable *Haemophilus influenzae* in invasive and mucosal respiratory diseases

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Non-typeable *Haemophilus influenzae* (NTHi) is a common commensal of the human respiratory tract mucosa. It is a fastidious Gram-negative coccobacillus that resides exclusively in the human host. To date, NTHi infection has been accepted largely as opportunistic and to occur particularly in cases in which the host's immune status or physiological barriers have been compromised, for example in acute exacerbations of chronic obstructive pulmonary disease in adults and otitis media (OM) in young children. However, there is increasing evidence that, despite the absence of a polysaccharide capsule, NTHi causes significant invasive disease as well as mucosal respiratory infections in subjects without predisposing risk factors. In both developed and developing countries, NTHi may cause septicaemia and meningitis in children at a prevalence at least equal to that due to a single, major *Streptococcus pneumoniae* serotype. In developed countries, especially in the post *H. influenzae* vaccine era, it is difficult to estimate the current disease burden, although some countries have suggested an increasing number of cases of invasive disease reported in the literature. Maternal genitourinary tract infection has been identified as a source of highly fatal, early onset neonatal NTHi septicaemia. The most significant mucosal infections known to be caused by NTHi are OM, sinusitis and conjunctivitis. Approximately one-third of the bacterial isolates from OM and sinusitis are NTHi. In the post-PCV-7 era, a proportionate increase in the frequency of recovery of NTHi from mucosal isolates has been demonstrated in children and adults in the United States. Furthermore, several lines of evidence suggests NTHi may also play an important role in paediatric community acquired lower respiratory infections, especially in children with recurrent and persistent bronchitis & pneumonia. Further studies are warranted to understand the public health importance of NTHi, as a pathogen.

This review suggests that NTHi, as a cause of invasive disease, has been underestimated and that there is an important invasive disease burden. NTHi appears to be more commonly responsible for mucosal respiratory infections than previously recognized.

S179 Clinical implications related to NTHI: from treatment to potential prevention

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Guidelines on the treatment of respiratory tract infections (RTI) may need to be re-evaluated considering the increasing importance of non-typeable *Haemophilus* influenza (NTHI).

- Reports comparing the prevalence of bacterial pathogens in acute otitis media and acute bacterial sinusitis in children prior to and after the introduction of the 7-valent conjugated pneumococcal vaccine (PCV7) show replacement of *Streptococcus pneumoniae* vaccine types with non-vaccine types. They also show an increased prevalence of NTHi.
- Recent reports confirm that NTHi are associated with recurrent and protracted morbidity.
- There are signs that the β-lactamase-dependent β-lactam resistance in NTHi is being replaced by a non-β-lactamase-dependent resistance mechanism that may render the use of current first-line treatment antibiotics, such as amoxy-clavulanic acid and second-generation cephalosporins, ineffective.

Worldwide, β-lactamase production accounts for the majority of *H. influenzae* ampicillin resistance. Mutations in penicillin-binding proteins are another β-lactam resistance mechanism. These *H. influenzae* strains are called β-lactamase-negative, ampicillin-resistant or BLNAR. BLNAR strains are less susceptible to ampicillin but also to amoxy-clavulanate and to second- or third-generation cephalosporins.

BLNAR strains were, until now, rare and increases in minimum inhibitory concentrations (MIC) were limited, making use of aminopenicillins in high doses still warranted. This situation is changing. Most notably, for example, BLNAR strains have increased significantly in Japan. Similarly, in some European countries (Spain and France), an increasing prevalence of BLNAR is also seen. Some of these strains have increased MIC values that make treatment with aminopenicillins or second- or third-generation cephalosporins ineffective.

A further increase of BLNAR strains might challenge our current guidelines for treatment of RTI that advocate use of amoxy-clavulanate or second-generation cephalosporins as first-line treatment options.

Based on the observed effects of PCV7, the possible clinical implications of new vaccines should be explored.

S180 The need for surveillance: a call for action

M.P. Slack (Abingdon, UK)*

In order to monitor the impact of Hib vaccination on the epidemiology of invasive *Haemophilus influenzae* disease, an international collaboration

was established in 1996. By 2006, 28 countries had responded and provided national surveillance data on invasive *H. influenzae*.1 By 2000, 14 of these countries had incorporated the Hib conjugate vaccine into their infant immunization schedules and routinely serotyped all clinical *H. influenzae* isolates. A total of 10,081 *H. influenzae* infections were reported between 2000 and 2006;2 of these, 2836 (28%) were due to Hib and 4466 (44%) to non-typeable H. Influenza (NTHi). It was found that the incidence of NTHi was almost twice that of Hib (0.28 versus 0.15 per 100,000 cases) and that both Hib and NTHi infections were more common in infants and the elderly. Moreover, the incidence of NTHi was much higher than Hib in the first month of life (11.4 versus 1.2 per 100,000 cases) and infections were more likely to occur in the first week of life, suggesting vertically acquired infection. Overall, however, the median age at disease was higher for NTHi infections than for Hib (58 years versus 5 years, $p < 0.0001$). NTHi infections were also associated with: a higher case fatality ratio (CFR; 366/3172 [11.5%] versus 88/2005 [4.4%]; $p < 0.0001$), particularly among infants (17.4% versus 2.9%; $p < 0.0001$); and a higher proportion of NTHi infections among women of child-bearing age. In addition, one-third of reported meningitis cases were due to NTHi. The CFRs for Hib and NTHi meningitis were similar in EU-IBIS data (4.1% versus 4.2%) but in England and Wales, the CFR was much higher for NTHi meningitis (17% versus 5%). The reasons for this are unclear. In conclusion, there is a need for continued, large-scale, clinico-epidemiological surveillance to collect data on clinical presentations of NTHi, risk factors, management and NTHi-attributable death.

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Emerging trends in fungal infections

K182 Emerging trends in fungal infections

C. Lass-Flörl (Innsbruck, AT)*

The frequency and diversity of serious fungal infections does increase and severely immunocompromised patients are particularly vulnerable to infection from moulds and yeasts. *Candida* and *Aspergillus* species are the predominant pathogens. *Candida* bloodstream infections are associated with high morbidity and mortality in both neutropenic and non-neutropenic critically ill patients. Risk factors associated are diverse and include exposure to broad spectrum antimicrobial agents, mucosal colonization, indwelling vascular catheters, surgery and cancer chemotherapy. Differences in geographical epidemiology are emerging, in particular regarding a shift towards non-*albicans* species. This shift has been correlated with routine fluconazole administration. *Aspergillus* spp. can cause a wide spectrum of diseases in humans, including allergy, superficial infection, and invasive disease. Invasive aspergillosis has emerged as a leading cause of morbidity and mortality in immunocompromised patients. The most important species are *Aspergillus fumigatus* followed by *Aspergillus flavus*.

Contemporaneously, infections with rare molds are on rise. Zygomyzetes, Trichosporon, *Fusarium*, Alternaria, Pseudallescheria and dematiaceous fungi are recognized more frequently. The emergence of these organisms is multifactorial and can be related to more intense immunosuppression, the prolonged survival of patients, and the selective pressure of broad spectrum antifungal agents used for prophylaxis or therapy. Among these rare mould infections, the zygomycetes are the most commonly encountered, and appear to be associated with the use of voriconazole. *Aspergillus terreus*, a species that is resistant to amphotericin B, and less frequently, *A. ustus* and *A. lentulus,* have been noted increasingly as causes of invasive aspergillosis in some tertiary care centres. *Scedosporium* with innate resistance to many antifungal agents have emerged as major causes of disseminated infections, followed by infections due to *Fusarium* species. Dematiaceous, or brown-black, fungi, most often associated with chronic localised infections, are now increasingly reported as a cause of disseminated infection in immunosuppressed hosts.

Watchful surveillance, rapid detection of disease, adequate treatment and effective control measures are highly warranted in the optimal management of these difficult to treat fungal infections.

Impact of the commensal flora on health and diseases

K183 Impact of the commensal flora on health and diseases

L. Engstrand (Stockholm, SE)*

Our adult bodies harbour approximately ten times more microbial cells than human cells and their genomes (the microbiome) endow us with physiological capacities that strongly influence our well-being. However, our microbiome is largely unexplored and there is a great need to increase our understanding of the interactions between our human and microbial genomes. The sequencing of the human genome constituted a starting point in the understanding of human biology at a global scale, yet today there is a growing agreement that human health and disease cannot be understood without considering the microbial communities. Now when the human microbiome project has been launched a number of international consortiums are starting up with efforts to explore the role of the human microbiota in health and disease. Many lines of evidence suggest a role for both commensal and transient microbes in the status of human health as well as for the risk of contracting certain diseases. During the last years molecular microbiology has revolutionized the landscape of microbiology and will continue to do so by providing new solutions for microbe identification and characterization. Next-generation sequencing has opened up new areas of research for people in the field of intestinal, skin, oral cavity and other microbiomics. The high-throughput sequencing platforms we now have access to will hopefully also help to increase our understanding of host-bacteria interactions, immune maturation and mechanisms behind chronic disease development. An interdisciplinary approach must be taken on comprising medical, epidemiological, computational, and biotechnology expertise focusing on understanding the human microbial communities and their effect on human health. Application of different "omics"-methods and computational systems biology methods to unique biobanks will be required in order to map out the human microbiome as well as the human cellular machinery interacting it.

ESBL-producing *E. coli* in the community

S185 How big is the threat in the outpatient setting?

N. Woodford (London, UK)*

E. coli with CTX-M enzymes are globally the most prevalent ESBL producers. They are often isolated from urines of patients attending general practice, but there are few data to assess accurately the extent of the community burden. The prevalence of ESBL producers in faeces from healthy people is typically <5% in Europe. In a recent multicentre study of non-hospitalized patients with infections, one third of the ESBL producers (mainly *E. coli*) were from those with no recent health care contact (Ben-Ami et al. Clin Infect Dis. 2009;49:682). In the UK, ESBL-producing *E. coli* cause c. 2500 cases of bacteraemia p.a., and may be estimated to cause c. 50,000 urinary tract infections p.a. Many belong to the globally-disseminated O25:H4-ST131 uropathogenic clone and have CTX-M-15 ESBL, though CTX-M-3 is equally common in this clone in Belfast, a city where the ST131 clone is present in the faeces of 40% of nursing home residents. CTX-M-15 ESBL is associated with IncFII multi-resistance plasmids, while CTX-M-3 in Belfast is encoded on IncI1 plasmids. These plasmids cannot readily be lost even in the absence of antibiotic selective pressure, since they encode multiple 'addiction' systems. Hence ESBL producers may serve as long-term community reservoirs of resistance genes. Foreign travel may also be associated

with gut colonization by ESBL-producing isolates, and the ESBL present often reflects the type most prevalent in the countries visited. Food remains an under-explored potential source for ESBL-producing *E. coli*. Raw chicken has been sampled in the UK, with CTX-M group 2 and 8 ESBLs found in meat imported from South America; these types account for <1% of ESBLs from clinical infections. There are currently no data to suggest wide presence of CTX-M-15 ESBL in foodstuffs; it may be found in *E. coli* from animals, but the strains are usually distinct from the dominant human clinical types. ESBL producers are often multi-resistant. Carbapenems are the drugs of choice for serious infections, but resistance may emerge in strains with reduced permeability, as observed in a UK nursing home resident who had no recent hospitalization or carbapenem exposure. Carbapenemase-producing *E. coli* are rare, although isolates with NDM-1 metallo-carbapenemase in addition to CTX-M-15 and acquired AmpC enzymes give cause for concern lest they become as prevalent as those with 'traditional' CTX-M-15 enzyme, or follow them into the community setting.

S186 How does changing epidemiology alter our management and prevention strategies?

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Since 2000 *Escherichia coli* producing CTX-M enzymes (especially CTX-M-15) have emerged worldwide as important causes of community-onset urinary tract (UTIs) and blood stream infections due to extended-spectrum β-lactamase (ESBL) producing bacteria. Molecular epidemiology studies suggested that the sudden worldwide increase of CTX-M-15-producing *E. coli* is mostly due to a single clone named ST131 and that foreign travel to high-risk areas such as the Indian subcontinent might play in part a role in spread of this clone across different continents. The carbapenems are widely regarded as the drugs of choice for the treatment of severe infections due to ESBL-producing Enterobacteriaceae Empiric antibiotic coverage for these resistant organisms should be considered in community patients presenting with sepsis involving the urinary tract especially in patients with certain risk factors such recent antibiotic use, residence in a long-term care facility, recent hospitalization, males older than 65 years and recent travel to a high-risk area. Failure to initiate appropriate antibiotic therapy from the start appears to be responsible for higher patient mortality. Studies from different parts of the world shows that that up to 5 percent of the population in the community can be rectally colonized by ESBL-producing Enterobacteriaceae but the large scale introduction of these bacteria into the hospital setting has not yet been demonstrated. However higher rates of colonization with ESBL-producing Enterobacteriaceae are present in household contacts of patients with infections caused by ESBL-producing Enterobacteriaceae, residents from nursing homes or long-term care centers. Preventing the spread of these bacteria and managing infections caused by ESBL-producing *E. coli* from the community remain difficult and controversial issues.

The integrated diagnostic platform versus the stand-alone microbiology laboratory

S188 The integrated diagnostic platform

J. Van Eldere (Leuven, BE)*

Clinical microbiology is subject to rapid evolution due to the need for rapid and more complex diagnostics, the increasing difficulty in recruiting trained personnel as well at the medical as at the technical level, the need for proven cost-efficiency and the increasing overhead costs associated with quality control. On the other side, there are new opportunities following the introduction of new techniques such as PCR, MALDI-TOF and in particular the availability of automated inoculation and plate reading systems to replace manual labour.
Reactions to these challenges differ, ranging from outsourcing to non-hospital based specialized microbiology labs to various forms of cooperation between smaller labs. This cooperation can exist in centralizing a part of or almost all activity in a central hospital-based lab or can be a network with a division of labor between collaborating labs. All these solutions share one factor. By increasing size, they seek to increase cost-efficiency and facilitate the introduction of new, specialized and expensive techniques. Outsourcing is often rejected because it risks reducing microbiology to a purely analytical activity with little microbiologist-dependent added-value. Concentrating smaller microbiology labs in one central lab or networking may not always be possible for practical reasons and will again increase the distance between clinical microbiologists and some of the microbiological tests.
A solution that can assure a sufficient lab size even in medium-sized hospitals without the need for collaboration with other labs or outsourcing is integration of the microbiology lab with other pathology labs such as clinical chemistry or clinical hematology. This kind of integration can combine economy of scale with conserving the microbiological activity within the hospital and thus the close link between microbiology and infectious disease and infection control specialists. Integration of laboratories in its simplest form can exist in sharing of machines and lab space. It can also lead to an integration of all diagnostic activity according to common workflows. An example is an integrated unit for molecular testing performing all nucleic acid based testing; microbiological, hematological but also human genetics or forensic medicine. This integrated lab demands new ways of organizing lab activity. Essential to the integrated lab is a separation of medical and technical expertise and medical and organizational responsibility.

EBV, CMV and virus hepatitis in solid-organ transplant recipients

S189 EBV and CMV-specific immunity

M.K. Brenner (Houston, US)*

Following allogeneic stem cell transplantation, patients have profound and prolonged immunosuppression. This is most severe after HLA mismatched transplants that require extensive T cell depletion to prevent graft-versus-host disease, and in recipients of cord blood transplants, which contain few memory T-lymphocytes. The consequence is a high morbidity and mortality from infection and disease relapse. We have been developing two approaches to repopulate the immune system following transplantation to reduce both types of risk. Our first approach is to isolate viral and fungal antigen reactive T lymphocytes from donor blood and expand them *ex vivo* before infusing them into the stem cell recipients. We have generated T lymphocytes that are specific for common viruses such as Epstein–Barr virus, cytomegalovirus, and adenovirus, and engineer them to expand efficiently *in vivo*. They have proved effective at preventing and treating serious virus illness in the target patient populations and we are now extending this approach to the treatment of fungal disease. Our second approach is to remove alloreactive T cells from donor PBM and infuse these depleted T cells to produce broad immune reconstitution. As a further safety measure to prevent GvHD from the infused cells, we incorporate a new suicide gene composed of a modified caspase 9 that can be activated by a small molecule drug. Early clinical results show high safety and efficacy of the approach.
We have also developed tumor-specific cytotoxic T lymphocytes, using both native and chimeric receptors directed to tumor associated antigens. We express the chimeric receptors in virus specific T lymphocytes, thus gaining the benefit of both an antiviral and an antitumor response from the same infused T-cells. These approaches appear to be extremely cost effective and to have a high margin of safety.

S190 Hepatitis E: monitoring and treatment

J. Izopet (Toulouse, FR)*

Hepatitis E virus (HEV) infection is now considered as an emerging infectious disease in industrialized countries. HEV genotypes 3 and 4

are widely distributed in animals and there is growing evidence that hepatitis E is a zoonotic disease transmitted directly or indirectly from animals to humans. It has been shown recently that HEV can lead to chronic infection in solid-organ transplant recipients. Liver fibrosis progression in this population can be very rapid, leading to liver cirrhosis within a few years after infection.

Virological tools are crucial not only for diagnosis but also for determining the incidence of HEV infections in solid-organ transplant recipients. Although the sensitivity of EIA tests for detecting anti-HEV antibodies among immunocompromised patients may be poor, the performances of the anti-HEV IgM assays appear to be useful for identifying a recent HEV infection. However, detection of HEV RNA in blood and stools by PCR remains the gold standard.

In a prospective cohort of 38 solid organ transplant recipients with acute hepatitis E, 58% developed a chronic infection. Two main HEV genotypes were identified by sequencing the ORF2 region, genotype 3f and 3c. The concentrations of HEV RNA in plasma at the acute phase in patients who cleared the virus were similar to those who developed a chronic infection. By contrast, the acute phase aminotransferase activities were higher in the patients who cleared the virus.

When possible, reduction of immunosuppressive drugs targetting T-cells in chronically infected patients must be considered as a first-line therapeutic option in order to obtain HEV RNA clearance. Recent data also indicate that pegylated interferon and ribavirin induce biochemical and virological responses in selected populations.

In conclusion, HEV infection must be considered in the differential diagnosis of hepatitis in solid-organ transplant patients. Improved information and prophylactic measures are needed to reduce the risk of virus transmission and chronic liver disease.

Impact and cost-effectiveness of MRSA screening

O195 Screening procedures have a significant impact on incidence densities of methicillin-resistant *Staphylococcus aureus* in German intensive care units

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Objectives: To evaluate the incidence densities (IDs) of methicillin-resistant *Staphylococcus aureus* (MRSA) and screening and control measures in intensive care units (ICUs) participating in the German Nosocomial Infection Surveillance System (KISS).

Methods: The surveillance module for multidrug-resistant bacteria in ICUs (MDR-KISS) collects data on all MRSA cases in participating ICUs with the aim to provide a national reference and a tool for monitoring and evaluation of infection control management. The median IDs of MRSA cases per 1000 patient-days (pd) with the interquartile range (IQR) are calculated for all MRSA cases and for the subcategories of imported and ICU-acquired cases from the pooled data of all ICUs as a reference for comparison with local MRSA IDs.

In 2008 a detailed questionnaire regarding ICU structure, microbiological diagnostics, MRSA screening procedures, MRSA control measures, infection control education and implementation of surveillance was sent to all participating ICUs. The association between questionnaire results and high MRSA IDs (>75% percentile) was evaluated by logistic regression analysis.

Results: 186 ICUs with 298631 patients and 1064246 pd had submitted data on 4730 MRSA cases for 2007–2008 and had completed the questionnaire. Median MRSA IDs in these ICUs were 3.23 (IQR 1.19–5.68), 2.05 (IQR 0.62–4.22) and 0.61 (IQR 0.14–1.35) per 1000 pd for all cases, imported and ICU-acquired cases, respectively. Routine screening for MRSA was performed in 142 (76.3%) ICUs, with conventional MRSA detection methods used in 74 ICUs (39.8%), PCR-based screening in 11 ICUs (5.9%) and both methods in 57 ICUs (30.6%). The most relevant screening procedures and control measures are shown in table 1. Of all variables derived from the questionnaire only the variables general admission screening of all patients (odds ratio 8.2, $p < 0.001$) and screening of admitted patients at regular intervals (odds ratio 4.9, $p < 0.001$) were independently associated with high MRSA IDs.

Conclusion: Considerable differences exist in MRSA incidence densities, screening and control measures in German ICUs. MRSA screening procedures have the most important impact on MRSA incidence densities. ICUs decide to perform a general admission screening probably because they have realized their MRSA problem.

Table 1. MRSA screening and control measures in 186 German intensive care units

	No. (%) of intensive care units
Computerized MRSA alert system	134 (72.0)
Screening of known carriers	118 (63.4)
Screening of contact patients	113 (60.8)
Screening of high-risk patients	104 (55.9)
General admission screening of all patients	51 (27.4)
Screening of admitted patients at regular intervals	39 (21.0)
Isolation of MRSA carriers in single rooms	174 (93.5)
Decolonisation with mupirocin ointment/antiseptic washing	173 (93.0)

O196 How many patients do you need to screen for methicillin-resistant *Staphylococcus aureus* to find a positive? Results from a screening programme in a UK teaching hospital

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Objectives: Screening for methicillin-resistant *Staphylococcus aureus* (MRSA) became mandatory in the UK for all elective and day case admissions from April 2009 and will be mandatory for all emergency admissions from April 2011. Data to assess the effectiveness of this strategy at an individual hospital level are lacking. We analyzed our screening programme data to determine the number needed to test (NNT) to detect a colonised patient across a range of clinical specialties, and develop a risk assessment strategy for MRSA screening.

Methods: MRSA screens of nose and groin were performed using chromogenic agar, with additional sites such as wounds and catheters included when present. In our hospital, MRSA screening data were resolved prospectively to individual patient level as opposed to a commonly utilized approximation using matched census (admission) data. Screening data were mapped to a patient administration system database containing relevant speciality codes. Mismatches where no screening results existed for individual patients were investigated weekly and included identification of patients in specific categories excluded from screening. Screening efficiency by specialty was fed back weekly to clinical teams to drive improved performance. Rates of MRSA positivity and NNT to detect a positive patient were determined for each specialty.

Results: For the 12 months from October 2008, the laboratory performed >160,000 MRSA swabs on 51,855 individuals costing 600,000 Euros. Screening efficiency for elective admissions improved from 60% to 99% (2228/2249) and for emergency admissions from 76% to 97% (2996/3077). Overall, 1.2% of hospital admissions were MRSA positive, with 1.6%, 0.7% and 0.6% being MRSA positive in emergency, elective and day case cases respectively. The highest MRSA positivity rates were seen in emergency cases in hepatology (4.3%), interventional radiology (3.7%) and vascular surgery (3.3%). 1.5% of elective vascular surgery patients were MRSA positive. The lowest MRSA positivity rate was in day case orthopaedics where 1,006 patients were screened without detecting a single positive patient. NNT to detect a positive ranged from 24 to >1,006 depending on clinical speciality and admission type.

Conclusions: Accurate data on results of MRSA screening can be used to assess both risk for individual patient admissions and the cost effectiveness of screening in specific patient groups, and to enhance the efficiency of the screening process.

O197 Long-term control of endemic methicillin-resistant *Staphylococcus aureus* in a tertiary centre: a segmented regression analysis of a stepwise approach

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Objectives: We evaluated the long term efficacy of several bundles of control measures against endemic methicillin-resistant *Staphylococcus aureus* (MRSA) in a 1000-bed tertiary hospital.
Methods: A cuasi-experimental study of interrupted time series was performed. The outcome variables were hospital-wide bimonthly rates (cases per 1,000 patient-days) of nosocomial colonisation/infection and bacteraemia due to MRSA from January 2005 to December 2008. The interventions were as follows: Intervention 1 (In1), implemented in January 1997: contact precautions for MRSA patients detected by clinical samples; intervention 2 (In2), implemented in January 1999: active surveillance of MRSA colonisation of patients and healthcare workers (HCW) in wards with ongoing MRSA transmission as suspected by the analysis of clinical epidemiologic data; and intervention 3 (In3), implemented in January 2001: active surveillance of readmitted patients and patients admitted from other centers. For the analysis, segmented regression was used. The following model was adjusted: Y = b0 + b1 x time + b2 x In1 + b3 x time after In1 + b4 x In2 + b5 x time after In2 + b6 x In3 + b7 x time after In3 + e, where b1=baseline time trend, b2=level change after In1, b3=trend change after In1, b4=level change after In2, b5= trend change after In2, b6=level change after In3, and b7=trend change after In3.
Results: The rate of colonisation/infection and bacteraemia in the preintervention period (1995–1996) was 0.56 and 0.10, respectively. Neither the level nor the trend chenged after In1. Rates were significantly reduced to 0.28 and 0.04 after In2, and to 0.07 and 0.02 after In3, respectively, and were kept in those levels since 2008. There was no significant changes in the rate of bacteraemia due to methicllin-susceptible *S. aureus*. The results cannot be explained by changes in the case-mix or antibiotic consumption. The segmented regression model is shown in the table.
Conclusion: Sustained control of endemic MRSA in tertiary centres is possible by active screening of patients and HCW, contact precautions, and control of high risk patients at hospital admission.

	MRSA colonisation/infection		MRSA bacteraemia	
	Coefficient (95% Cl)	P	Coefficient (95% Cl)	P
β_0	0.58 (0.49; 0.67)	<0.001	0.10 (0.076; 0.12)	<0.001
β_1	–0.004 (–0.16; 0.008)	0.4	0.001 (–0.003; 0.004)	0.75
β_2	0.03 (–0.08; 0.14)	0.5	–0.018 (–0.050; 0.014)	0.2
β_3	0.001 (–0.016; 0.018)	0.8	0.002 (–0.003; 0.006)	0.4
β_4	0.06 (–0.05; 0.18)	0.2	–0.05 (–0.08; –0.02)	0.002
β_5	–0.04 (–0.06; –0.02)	<0.001	–0.006 (–0.010; –0.001)	0.01
β_6	0.07 (–0.01; 0.16)	0.04	0.002 (–0.022; 0.026)	0.8
β_7	0.04 (0.03; 0.05)	<0.001	0.003 (0.000; 0.006)	0.05

O198 Diagnostic yield and financial consequences of anatomic sites tested using rapid diagnostic tests for methicillin-resistant *Staphylococcus aureus*

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Objectives: The Dutch MRSA screening strategy requires sampling of multiple sites and with rapid diagnostic testing (RDT) conventional cultures are mandatory as back up. We determined cost-effectiveness of a less extensive screening regime using RDT without back up cultures in a prospective multi-center study.
Methods: All patients at risk of MRSA colonization and fulfilling the criteria for pre-emptive isolation in 14 hospitals between 12/05 and 06/08 were eligible. In addition to conventional cultures, BD GeneOhmTM MRSA PCR ('IDI', BD Diagnostics) or the Xpert MRSA assay ('GeneXpert', Cepheid) was performed directly on patient material. In a nested cohort study the chromogenic agar MRSA-ID (bioMérieux) was tested. Cost-efficacy is determined assuming isolation measures would have been based on single site testing of the nares without back up cultures. Isolation of MRSA at any site tested using broth enrichment culture was the gold standard.
Results: 1764 patients (mean 48 years, 56% male) were enrolled. 3.3% carried MRSA: after hospitalization abroad 1.6% (20/1225), contact with pigs 15.0% (29/193) and contact screening related to MRSA positive patients 2.8% (8/289). The sensitivity of screening of the nares using IDI PCR, GeneXpert and MRSA-ID was 69.2%, 56.3% and 61.5%, respectively with negative predictive values >98.4% for all tests. Costs per patient tested were €56.62, €70.92 and €8.06 for IDI PCR, GeneXpert PCR and MRSA-ID, respectively. Isolation days avoided were 2.3%-5.2% higher with single site testing compared to multiple site testing because of higher specificity (and lower sensitivity). Test costs per isolation day avoided were €25.00, €34.48 and €4.91 for IDI PCR, GeneXpert PCR and MRSA-ID respectively. As compared to "nares-only" multiple site testing prevented respectively, 4 and 6 false negative cases using IDI PCR (4/853, 0.5%) and GeneXpert PCR (6/911, 0.7%) at the additional costs of respectively €148.55 and €161.78 per patient. Chromogenic agar testing of multiple sites prevented 4 FN results (4/428, 0.9%) at no additional costs.
Conclusion: Although the recommended multiple site sampling strategy has a higher sensitivity than "nares-only" screening without back up cultures, our results demonstrate that in a low endemic setting the benefits of such a strategy are limited, and the costs are high. Financial consequences of the missed MRSA patients have to be determined to draw definite conclusions.

O199 Modelling the cost-effectiveness of screening and decolonization in the control of methicillin-resistant *Staphylococcus aureus*

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Objectives: To use transmission dynamic models to assess the cost-effectiveness of defined screening and decolonisation strategies in the control of methicillin-resistant *Staphylococcus aureus* (MRSA).
Methods: We developed a dynamic transmission model of MRSA in intensive care units (ICUs) to evaluate the effectiveness and cost-effectiveness of screening and topical decolonisation (both nasal ointment and body wash-based antiseptic protocol). The model was parameterized using evidence from multiple sources (data, literature and expert opinion) including full parameter uncertainty. Thirteen strategies were compared, including decolonisation of all admissions, decolonisation of patients identified as MRSA positive through screening (comparing conventional cultures, chromogenic agars and polymerase chain reaction (PCR)-based techniques) and targeting interventions to high risk patients. Incremental costs and health benefits of the alternatives were evaluated under different settings of prevalence, proportion of high risk patients and ICU size.
Results: Compared to a baseline strategy of decolonisation of clinical infections only, all other strategies were cost-saving and gave increased health benefits. Decolonisation of all patients was the most cost effective option, saving £60,000/quality adjusted life year (QALY) gained. For screening, and decolonisation of identified MRSA positives, PCR had the greatest ability to reduce MRSA transmission (giving a 94% reduction in infections per 100 admissions, compared to 64% with conventional culture). Including parameter uncertainty, the best decision depended on the decision maker's willingness to pay for health benefits: at a value of £20,000–30,000 per QALY, admission screening with PCR combined with decolonisation of identified positives was the best strategy. However, there was uncertainty in this decision, reflecting parameter uncertainty,

and the expected value of perfect information on the parameters was high (~£10,000).
Conclusion: With no microbial resistance to decolonisation agents, all decolonisation strategies were cost-effective, particularly decolonisation of MRSA positive admissions identified through PCR screens. These models allow uncertainty in decision making to be quantified and highlight parameters on which more information is needed to ensure cost-effective decisions, in this case the probability of progressing from colonisation to infection for decolonised and 'un-decolonised' patients.

Future high-throughput microbiology laboratory

S204 I believe in mass-spectrometry

X. Nassif (Paris, FR)*

MALDI-TOF mass spectrometry: a revolution in the identification of pathogens in clinical laboratories. In the management of bacterial infections, identification of the pathogen following growth remains essential to propose as soon as possible the most appropriate treatment before the availability of antibiotic susceptibility. The strategy for most common pathogens requires Gram staining, the results of simple tests such as determination of oxidase and catalase activity and appropriate phenotypic tests using commercial identification kits and/or automated systems. A new proteomic strategy is progressively changing this strategy by identifying bacterial or fungal species grown on plates within minutes. Matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) of intact bacteria yield reproducible spectrum depending upon strain or species. Using whole viable bacteria we describe here the application of MALDI-TOF-MS to the identification of bacterial pathogens isolated in routine clinical microbiology laboratories. Our aim was, once a primary isolate of a bacterium has grown onto a plate, to identify spectral prints, in the MALDI-TOF spectrum, that can be used to recognize the genus or the species. MALDI-TOF-MS was performed using bacteria obtained from one isolated colony. Over 400 clinically relevant bacterial species were selected. For each of these species one or several references strain was (were) selected to establish spectral prints. For each strain, only peaks that were conserved in the spectra of all 10 isolated colonies and with a relative intensity above 0.1 were retained, thus leading to a set of 3 to 30 selected peaks per strain. For each group of pathogens the database was validated using a set of isolates identified using mostly 16S RNA sequencing. The use of this strategy to identify bacterial species in clinical microbiology laboratories will be presented.

Climate change and parasitic infections in Europe

S206 Expansion of leishmaniasis and other parasitic diseases in Europe

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Objectives and Methods: To give an overview over climate change in Europe with some regional examples and the knowledge we have on its influence on the prevalence of parasitic infections with the use of review of literature.
Results: The northern part of Europe, the Arctic will have three times higher temperatures than the rest of the world due to climate change. We have a lot to learn from what is happening in the circumpolar area for adaptation strategies. Parasitic infections as *Leishmania*, Opistorchis, Echinococcosis multilocularis and alveolaris are expanding along with *Giardia intestinalis* as some examples. The impact will differ regionally.
Conclusion: Given the dynamis and the complexity of climate/sensitive infectious disease, particularly those transmitted by mosquitos or rodents Europe needs to develop and sustain surveillance and early warning systems with a regional focus.

Food for thought: tackling the problem of food-borne infection in the elderly

S209 The elderly patient as a sitting duck for food-borne infections: causes and consequences

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According to the United Nations, the world population of elderly will surpass that of children under age 15 by the year 2045. Health resources will shift with population growth; thus, measures to reduce health cost through preventive care are prudent. Illnesses from food-borne pathogens are avoidable if technologies are used to maximize safety of food and water, populations are educated in self-care, and medical therapies are advanced for early diagnosis and treatment. Since self-care strategies require alteration of usual cultural and habitual behavior, education efforts have limited success with the elderly. Why is it difficult to teach the elderly how to handle food safely and what are the consequences for failure to heed professional advice? Medically, when is a person elderly? When does the body begin senescence and susceptibility to opportunistic infections increase? The aging immune system is the primary reason for susceptibility to food-borne pathogens in the elderly. Natural, senescent immunity is exacerbated by the immune insufficiency of chronic disease and pharmacological therapies common in the population. Immune insufficiency associated with malnutrition becomes a causative factor as the physical and mental capacity of the aging individual deteriorates. Poor hygiene, failure to handle and store foods safely, and inability to distinguish between safe and tainted food may all be consequences of diminished mental or general health. For those in group housing, failure of care staff to heed food handling guidance could affect the health of multiple residents due to wide-spread exposure and immune insufficiency. Yet, acceptance of well-meaning advice to change poor health habits is limited due to lack of trust in the efficacy of the information, information that is contrary to cultural and traditional practices, or the failure to recognize and accept the decline of physiological and mental health. The challenge to the health professional that is providing food safety guidance is to recognize the social, health and mental barriers that control the ability of the individual to act on the advice. The goal is no food-borne illness in the world, but reality is that when preventative care is no longer possible, palliative care must take its place.

Paediatric infectious diseases

O215 Aetiology of community-acquired pneumonia in children during the influenza A (H1N1) outbreak

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Objective: In July 2009, a respiratory illness outbreak caused by influenza A virus (H1N1–2009) was identified in Caxias do Sul, Brazil. The aim of the study is to determine the impact of influenza A H1N1 2009 on the viral etiology of pneumonia among children admitted at General Hospital of Caxias do Sul from July 01 to August 31, 2009.
Methods: Retrospective medical chart reviews on the pediatric hospitalized patients diagnosed with acute respiratory infection between July and August 2009. We evaluated demographic data, clinical and laboratory findings. Nasopharyngeal aspirates for detection of respiratory viruses were analyzed by indirect immunofluorescence (IFI) and real-time polymerase chain reaction (RT-PCR).
Results: A total of 53 children were hospitalized for severe acute respiratory disease, according to the criteria established by the Health Ministry of Brazil. Most patients were male (60.3%) and had less than one year of age (35.8% with less than 6 months and 26.4% between 6 and 12 months). Comorbidities were identified in 9.4%. Fifteen patients were referred for the intensive care unit, and one third of them required mechanical ventilation. No deaths occurred. Six patients (11.3%) had

confirmed influenza A (H1N1) and in 27 children (50.9%) were identified respiratory syncytial virus (RSV) as the etiologic agent of pneumonia.
Conclusion: As in previous years, respiratory syncytial virus has remained as predominant agent in the etiology of viral pneumonia in spite of the outbreak of influenza A (H1N1) experienced in 2009.

O216 Norovirus infection in hospitalized Australian children

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Objective: Norovirus, previously the Norwalk agent, is a common cause of acute gastroenteritis. We determined the incidence and relative impact of norovirus in children with acute diarrhoea admitted to a tertiary paediatric hospital in New South Wales, Australia.
Methods: Faecal samples were collected from children presenting to our Hospital with episodes of acute diarrheal illness over a 12 month period (2007). The viral pathogens, rotavirus and adenovirus were detected using immunochromatography and norovirus was detected using enzyme linked immunosorbent assay.
Results: During this 12 month period, faecal samples were collected from 3962 children who presented with diarrhoea to our Hospital and viral pathogens were detected in 294 of these children who required hospital admission. 231 of 294 (78.5%) children with gastroenteritis required a hospital stay of one week or less, median 1 day and 212 had a single viral stool pathogen detected of which 63 (30%) were norovirus, 56 (26%) were adenovirus and 93 (44%) were rotavirus. Dual viral infections were detected in 19 (8%). Norovirus infections most commonly presented in spring (September-November, with an October peak) in infants (age less than 12 months). Of the 63 patients with a hospital stay greater than one week, 28 (44%) had norovirus infection, 16 were non-oncology patients who developed nosocomial infection after admission for another condition. The 25 oncology patients were older and had a longer hospital stay (median 11 days) than non-oncology patients and 7 (28%) had nosocomial infection due to norovirus.
Conclusion: We have demonstrated the impact of community-acquired norovirus gastroenteritis and the high frequency of nosocomial norovirus infection in a tertiary paediatric hospital. With the advent of rotavirus vaccination, norovirus may become the major viral pathogen causing gastroenteritis in Australian children.

O217 Impact of rapid PCR detection of enteroviruses in spinal fluid in children with meningitis

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Objectives: We hypothesized in this study that the use of a rapid PCR on enteroviruses in spinal fluid significantly reduces the duration of hospitalization, and use of antibiotics. We expected that these reductions would lead to lowering of hospital costs.
Methods: The study group comprises children admitted to the hospital from April 2009 until November 2009 with confirmed enterovirus meningitis. Since 2009 we performed the PCR on enteroviruses in spinal fluid in our hospital. The results are available within 3 hours. This study group was compared to a historical control group that consisted of children who were admitted in 2007 and 2008 with confirmed enterovirus meningitis. In the historical control group, results for enterovirus PCR tests were available after 3 to 7 days. We analyzed both groups for clinical and laboratory parameters, length of hospital stay, use of antibiotics and estimated overall costs.
Results: The study group and historical control group were comparable with respect to clinical and laboratory data. A significant difference was found between the groups in length of hospital stay ($p < 0.0001$). The mean duration was 6.7 days in the 2007–2008 group (range 2.3–41.0 days) versus 1.8 days in the 2009 group (range 0.8–4.55 days). The mean duration of use of antibiotics was also significantly reduced ($p < 0.0001$) from 4.48 days (range 1.3–12.8 days) to 0.75 days (range 0.1–1.5 days). Overall costs were 2900 euro lower per patient in the 2009 group.
Conclusion: Our data show that in children with enterovirus meningitis the use of a rapid PCR results in a significant reduction of hospital stay and duration of antibiotic treatment. Subsequently, it also leads to an important reduction of hospital costs. The rapid enterovirus PCR is an important diagnostic tool in daily management of children with meningitis.

O218 Predictive model for diagnosis of neonatal sepsis

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Early diagnosis of neonatal sepsis is essential to prevent severe complications and avoid unnecessary use of antibiotics.
Objective: To develop a predictive model for the diagnosis of neonatal sepsis.
Methods: This case–control study was conducted in QSNICH, Bangkok. Data were derived from the medical records of 45 sepsis and 135 non-sepsis neonates for early sepsis, and 52 sepsis and 156 non-sepsis cases for late sepsis, during the period 1 October 2004–30 September 2007. Potential predictors consisted of risk factors, clinical conditions, laboratory data, and treatment modalities. The models were developed based on multiple logistic regression analysis.
Results: The incidence of early and late proven neonatal sepsis was 1.27 and 1.46%, respectively. For early neonatal sepsis, the equation and score consisted of 5 variables: length of stay pre-sepsis, poor feeding, abnormal oxygen saturation (<92%), thrombocytopenia (<150,000/mm^3), and leucopenia (<5000/mm^3). For late neonatal sepsis, the model had 6 variables: poor feeding, abnormal heart rate (outside the range 100–180 x/min), abnormal temperature (outside the range 36–37.9ºC), abnormal oxygen saturation, abnormal leucocytes(according to Manroe's criteria by age), and abnormal pH (outside the range 7.27–7.45). The area below the ROC curve were 87.8 and 95.5% for early and late neonatal sepsis, respectively. Validation used subsets of the original data-set, twice for each model, and produced areas below the curve of 82.2 and 86.2% (for the early group) and 96.3 and 93.6% (for the late group). For early sepsis, score 1 had a sensitivity of 73.3% and specificity of 84.4%. For late sepsis, score 2 had a sensitivity of 88.5% and specificity of 90.4%.
Conclusion: 2 predictive models were developed, one for proven early-onset and another for proven late-onset neonatal sepsis. Derivation and preliminary validation produced good results.

O219 Failure of oral colistin to prevent colonization with extended-spectrum β-lactamase-producing enterobacteria in newborns hospitalized at a neonatal intensive care unit

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Objectives: Colonisation and infection with extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL-E.) is an emerging problem at Neonatal Intensive Care Units (NICU). ESBL-E. often show additional antimicrobial resistance. Colistin is reported to be effective in treatment of infections with multiresistant enterobacteria as well as for selective digestive decontamination (SDD).
Over the last years several outbreaks of ESBL-E. colonisation occurred at our NICU.
Methods: For prophylaxis of necrotizing enterocolitis (NEC) all term and preterm born neonates hospitalised at the NICU of the Medical University of Graz routinely receive gentamicin orally (15 mg/kg/d). Patients are routinely screened at least twice a week for ESBL-E. in stool specimens. ESBL-E. colonised patients subsequently are cohorted. From May 2005 through September 2007, gentamicin was replaced by colistin (8 mg/kg/d) administered orally during ESBL-E. outbreak situations.
We retrospectively analysed all neonates colonised with ESBL-E. from May 2005 through September 2007 regarding influence of colistin on colonisation with ESBL-E.. Genetic relatedness of strains was assessed by using repPCR-technique.

Results: During the study period 3 ESBL-E. outbreaks were observed and 30 (2.02%) out of 1488 neonates have been colonised with ESBL producing *Klebsiella pneumoniae* (ESBL-Kp, n = 22) or *Klebsiella oxytoca* (ESBL-Ko, n = 8). 12 out of 22 pts. colonised with ESBL-Kp and 1 out of 8 pts. colonised with ESBL-Ko had received oral colistin at time of colonisation with ESBL-E. Four different clones of ESBL-Kp and 2 different clones of ESBL-Ko were isolated indicating occurrence of patient-to-patient transmission.
Conclusion: Despite administration of Colistin during outbreaks of ESBL-E. colonisation, additional colonisations (including patient-to-patient transmission) were observed. Thus, oral colistin (8 mg/kg/d) does not prevent colonisation with ESBL-E. Further analyses are needed to assess its usefulness for SDD.

Success stories about control of nosocomial antimicrobial resistance – yes, we can!

S222 KPC control (Israel)

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Containment of a nationwide outbreak of KPC-producing *Klebsiella pneumoniae* via a centrally-coordinated public health intervention.
Background: Since 2006, Israeli hospitals have faced a clonal outbreak of carbapenem-resistant *Klebsiella pneumoniae*, producing the serine carbapenemase KPC-3. Locally-implemented infection control measures in affected hospitals failed to contain spread. A nationwide intervention was launched to contain the outbreak and introduce a strategy to control future dissemination of antibiotic-resistant bacteria in healthcare facilities.
Methods: In March 2007, the Ministry of Health issued guidelines mandating physical separation of hospitalized carriers of carbapenem-resistant Enterobacteriaceae (CRE) and dedicated staffing, and appointed a professional task force charged with containing the spread of the epidemic strain. The task force paid site visits at acute care hospitals, evaluated infection control policies and laboratory methods, supervised adherence to the guidelines via daily census reports on carriers and their conditions of isolation, provided regular feedback on performance to hospital directors, and intervened additionally when necessary. During 2008, the intervention was extended to long-term care facilities, and in June 2008 national guidelines for active surveillance were issued. The primary outcome measure was the incidence of nosocomial CRE cases diagnosed by clinical culture in acute care hospitals.
Results: By March 2007, over 1200 patients were affected in the nation's acute care hospitals. Prior to the nationwide intervention, the monthly incidence of noscomial CRE climbed steadily, peaking at over 180 cases. Crude 30-day mortality was >30%. With the intervention, the continuous rise in incidence of CRE acquisition was halted, and by the end of the 14-month initial intervention period the number of new monthly cases was reduced to 46. Following the introduction of active surveillance guidelines, monthly incidence fell further, reaching a low of 24 as of October 2009. A direct correlation was observed between compliance with isolation guidelines and success in containment of in-hospital CRE transmission.
Conclusions: A centrally-coordinated public health intervention has succeeded in containing a nationwide outbreak of CRE in Israeli hospitals after local infection control measures failed. The intervention demonstrates the importance of strategic planning and national oversight in combating antimicrobial resistance.

MALDI-TOF in clinical microbiology

S224 Microbiological identification by routine use of MALDI-TOF

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The analysis of bacterial protein profile obtained after matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) emerged over the past years as a new method for the accurate identification of bacterial isolates. MALDI-TOD-MS protein profiling can be achieved within muinutes thus speeding the identification; it is a cheap technology including the decreasing cost for mass spectrometers; protein profiles and issued identifications are reproducible and robust; and new generation mass spectrometers are smaller machines easily implanted within the laboratory. All these parameters make MALDI-TOF MS the technology to be introduced in any modern microbiology laboratory, including the point-of-care laboratory.
Databases, the crucial part of the process, now comprise of most of the bacterial species routinely encountered in the microbiology laboratory. Despite the fact that some Gram-positive organisms remain "MALDI-TOF resistant" in part because of underlying taxonomic weaknesses and in part because of database weaknesses, MALDI-TOF is definitely the first line method for the routine identification of bacterial isolates in the 21 century, pushing biochemical profiling and even the gram staining off the laboratory towards the museum of bacteriology.

S225 Bacterial typing

T. Hain (Giessen, DE)*

Rapid identification and typing of *Listeria* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Listeria monocytogenes* is a food-borne pathogen that is the causative agent of human listeriosis, an opportunistic infection that primarily infects pregnant women and immunologically compromised individuals. Rapid, accurate discrimination between *Listeria* strains is essential for appropriate therapeutic management and timely intervention for infection control. A rapid method involving matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) that shows promise for identification of *Listeria* species and typing and even allows for differentiation at the level of clonal lineages among pathogenic strains of *L. monocytogenes* is presented. A total of 146 strains of different *Listeria* species and serotypes as well as clinical isolates were analyzed. The method was compared with the pulsed-field gel electrophoresis analysis of 48 *Listeria* strains comprising *L. monocytogenes* strains isolated from food-borne epidemics and sporadic cases, isolates representing different serotypes, and a number of *Listeria* strains whose genomes have been completely sequenced. Following a short inactivation/extraction procedure, cell material from a bacterial colony was deposited on a sample target, dried, overlaid with a matrix necessary for the MALDI process, and analyzed by MALDI-TOF MS. This technique examines the chemistry of major proteins, yielding profile spectra consisting of a series of peaks, a characteristic "fingerprint" mainly derived from ribosomal proteins. Specimens can be prepared in a few minutes from plate or liquid cultures, and a spectrum can be obtained within 1 minute. Mass spectra derived from *Listeria* isolates showed characteristic peaks, conserved at both the species and lineage levels. MALDI-TOF MS fingerprinting may have potential for *Listeria* identification and subtyping and may improve infection control measures.

S226 MALDI-TOF-MS of surface-associated and stable intracellular proteins for identification and resistance profiling of human pathogens

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Microbial classification and identification is today based largely upon comparative 16S rRNA sequencing analysis. There is, however, a desire to retain a polyphasic approach as microorganisms are far too diverse to be unequivocally delineated by a single method. Proteomics, in the form SDS-PAGE, MLEE or IEF profiles, have had a long history of applications and have shown excellent congruence with genomic methods such as DNA-DNA reassociation. The arrival of MALDI-MS and ESI-MS has made the study of the proteome so accessible that within less than a decade platforms are now available for clinical applications. Thus, the development of intact cell MALDI-TOF mass spectrometry is

approaching maturity and its uptake by diagnostic laboratories has been increasing steadily. Extensive databases eg AnagnosTec (>100,000 MS spectra), negligible sample preparation and rapid analysis have been the main reasons for its success. The peptides/proteins present in the MS spectra are either surface-associated on intracellular ribosomal proteins, consequently, at present, little information can be obtained using solely this approach to gain information on antibiotic resistance patterns. More in-depth analysis involving 1D, 2D and LC-MS-MS methods are required. Using such approaches, new information on antibiotic resistance mechanisms are becoming apparent and providing an excellent tool to elucidate complex mechanisms where genomic analysis alone cannot provide a solution.

S227 Yeast identification

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Identification of clinical yeasts is an important task in the mycological laboratory especially in view of a rising number of patients with respective opportunistic mycoses. Reliable species identification can be used as a first guidance for initiation of an appropriate antifungal therapy. Classical phenotypical identification of yeasts is often tedious and needs up to 3 days for optaining a reliable result. A further obstacle is the limited databases of commercially available systems and test results with variable outcome for a given species. Genetic identification by in situ hybridization or by sequence analysis is relative expensive in terms of consumables per test.

After initial introduction of mass fingerprinting for species identification of bacteria by Claydon et al. 1996 availability easy-to-handle instruments and equiped with sophisticated analysis software facilitated use of this technique also for identification of yeasts in the last years.

Qian et al. (2008) described the use a fixation step by using alcohol as an essential prerequisite for obtaining improved mass signatures in case of yeasts. In a comparative study Marklein et al. (2009) could show that usage of a commercial MALD-TOF MS-based microbial identification system (MALDI BioTyper System, Bruker Daltonics) was superior to a phenotypic based identification system in case of clinical yeasts. We could successfully demonstrate that addition of user defined reference spectra to this database facilitates a reliable discrimination of members of the *Candida parapsilosis* clade. Von Bergen et al. (2009) used this system for the otherwise difficult-to-achieve identification of potentially pathogenic Prototheca spp., a group of algae with a yeast-like cultural appearance. Of note, Marinach et al. (2009) demonstrate that MALDI MS is capable to estimate Fluconazole resistance in case of *C. albicans.*

In conclusion, MALDI-TOF MS fingerprinting turned out as a very promissing tool for reliable and rapid (hands-on time <5 min) identification of clincal yeasts with low costs for consumables per identification (<1 €). This approch was not so much hampered by influence of the morphology of the fungus and cultural conditions e. g. culture media and minor changes in incubation temperature when compared to MS-based identification of hyphomycetes. So this technique facilitates high through put and objective identification of yeasts when using a quality-checked database.

Infections in the elderly, new concepts

S228 HIV and aging

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Epidemiological data show that HIV-infected population is aging, mainly as a result of prolonged survival due to potent antiretroviral regimens that can successfully control viral replication, thus preventing AIDS-related events, but also of a late diagnosis of HIV infection in older patients. No specific guidelines have been developed for the clinical management of older HIV-infected patients but clinicians must take into account several factors that are peculiar of this population, like the presence of co-morbidities, the pharmacokinetics characteristics of the elderly, a possible higher incidence of drug-related toxicity and a different response to antiretroviral therapy.

In older HIV-infected population, morbidity and mortality still remain high as a consequence of an increased risk of non-AIDS related conditions compared to age-matched HIV-seronegative subjects. These conditions include cancers, neurocognitive disorders, cardiovascular, liver, kidney and bone diseases. Many factors contribute to non AIDS-related co-morbidities and they can be related to the population characteristics (e.g. higher prevalence of traditional health-related risk factors like diabetes, hypertension, hyperlipidemia and smoking), to antiretroviral drugs (e.g. association between long term exposure to protease inhibitors and higher risk of cardiovascular disease, tenofovir exposure and renal or bone dysfunction) or to the HIV-infection itself (e.g. association between age-related events and low CD4 cells count or higher levels of inflammation markers found in HIV-positive patients).

The pharmacokinetic characteristics of the elderly are peculiar, with a possible modification of drug absorption, distribution and hepatic or renal clearance that results in a high inter-individual pharmacokinetic variability. Moreover, interactions between antiretrovirals and drugs frequently prescribed for non HIV-related co-morbidities can further contribute to this variability. As a consequence, plasma drug levels can drop below effective concentrations promoting treatment failure or can become high resulting in toxicity.

Many data shows that, despite a high rate of virological suppression (usually attributed to high adherence to the therapy), older patients had a significantly slower CD4 cells count reconstitution than younger subjects. The causes for such finding could be due to several factors like the late diagnosis and treatment of HIV infection on the one hand, or the thymic involution and immunosenescence during aging on the other hand. Many studies indicated that suboptimal CD4 cells increase is associated to a higher risk of non AIDS-associated morbidity and mortality (for cancer, heart and liver disease) in this patient population.

In conclusion, as the prevalence of older HIV-infected patients is increasingly growing, additional research is needed to fully understand factors contributing to the peculiar evolution of HIV infection and associated co-morbidities in this age group, in order to optimize the clinical care of this population.

S230 Infectious disease outbreaks in nursing homes

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Advances in health care have conduced in developed countries to an aging population. Long-term care facilities (LTCF) are institutions which provide heath care to people unable to manage themselves in the community. Most residents are elder and have multiple comorbidities. Residents will stay at LTCF for months or years. Hence, comfort, dignity and socialization are important issues. In order to control costs, many post-acute patients who used to stay in acute care hospitals for a long time, are now currently transferred to LTCF. This situation has led to the necessity of invasive devices such as urinary and vascular catheters and feeding tubes. This fact increases the incidence of nosocomial infections, as does the one observed in hospitals. Several significant differences can be pointed out between hospitals and LTCF; most notably the nurse-patient ratio, the availability of microbiological cultures, laboratory and diagnostic equipment on site and the access to costly antibiotics. In addition there are few LTCF with infection control staff. Most of them lack of nosocomial infections surveillance programs and also of antibiotic policy strategies. In general, rates of colonization by multiresistant bacteria in residents of LTCF are higher than those observed in hospitalized patients. For example, some studies have shown that rates of methicillin resistant *Staphylococcus aureus* (MRSA) carriage among LTCF residents can reach 50%, while in hospitals are generally below 1–2%. Although it is possible to detect outbreaks, MRSA infections in LTCF are sporadic, related to pressure ulcers and generally less serious than those seen in hospitals. The prevalence of multidrug resistant Gram-negative (MDR-GNB) bacteria is also high among LTCF residents, particularly among those with faecal incontinence and antibiotic exposure. MDR-GNB is frequently

recovered from environmental samples and common areas providing opportunities for person-to-person transmission. *Clostridium difficile* continues to be an increasing problem in LTCF. It is the most common cause of non-epidemic acute diarrheal illness in LTCF. Outbreaks have also been recognized, particularly related to antimicrobial consumption. In conclusion, LTCF act as a reservoir of multi-resistant bacteria that can cause sporadic infections as well as indolent outbreaks. A multilevel approach, including antimicrobial policies and prevention transmission measures are needed to limit the impact of this problem.

S231 Herpes zoster: is there a role for vaccination?

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Herpes Zoster (shingles) is caused by reactivation of the varicella zoster virus. It is characterized acutely by a painful rash, and 10–20% of patients experience prolonged pain (postherpetic neuralgia), which in rare cases can last for years and be debilitating. Both the incidence and severity of zoster increase with age. Treatment options for zoster and post herpetic neuralgia are limited and costly. A shingles vaccine was licensed in Europe and the United States in 2006 following the completion of the Shingles Prevention Study, a randomized controlled trial of 38,546 healthy adults; vaccination decreased both the incidence and severity of zoster over a median of 3 years. Primary efficacy was measured by a burden of illness score which combined incidence, duration and severity of pain. The vaccine decreased burden of illness by 61%, but the effects varied by age. Efficacy in preventing acute zoster declined with age at vaccination, and there was minimal efficacy past age 80 years. Vaccination reduced the incidence of post-herpetic neuralgia at all ages by 66%. This was achieved by preventing acute zoster among patients aged 60–69 years, and attenuating disease in patients aged $\geqslant$70 years. Side effects were mild, but serious adverse events were more common in the vaccination group (1.9 percent vs. 1.3 percent, P = 0.03). Debate about the use of the vaccine revolves not around the effectiveness, but the cost-effectiveness, as the vaccine is expensive (€136 per dose). Published analyses have found incremental cost-effectiveness ratios ranging from €18,500 to €76,500 per quality-adjusted life year. The vaccine appears to be most cost-effective among 65- to 70-year-olds, as the incidence of disease in this age group is high, but vaccine recipients are still able to mount a satisfactory immune response. Despite recommendation by the Centers for Disease Control that all adults aged $\geqslant$60 years receive the shingles vaccine, uptake in the US has been slow. Barriers to vaccination include difficulties with vaccine storage, variable insurance coverage, and competing priorities for primary care doctors. Since licensure, the cost of the vaccine has increased by 30%. Moreover, studies of willingness-to-pay suggest that current vaccine pricing is not acceptable to patients. In conclusion, the shingles vaccine appears to be effective in reducing morbidity, but the cost is high and universal vaccination over age 60 years may not currently represent good value.

Rapid diagnosis and resistance testing in mycobacteria

O233 Improved sensitivity of rapid detection of *Mycobacterium tuberculosis* in clinical specimens by real-time PCR

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Objective: Our aim was to compare the use of DNA amplification by two polymerase chain reaction (PCR) tests for the detection of *Mycobacterium tuberculosis* directly in human respiratory and extrapulmonary specimens. For this purpose, the sensitivity and specificity of culture and smear diagnostics as well as the Cobas Amplicor PCR test and the Cobas TaqMan MTB real-time PCR test were compared in a trial study.
Methods: The PCR assays employed was the Cobas Amplicor *M. tuberculosis* test and the real-time PCR Cobas TaqMan MTB test (Roche Diagnostics, Switzerland), which use the 16S rDNA as the target template. Eight hundred and seventy-eight samples from 763 patients submitted to our clinical microbiology laboratory were processed by enriched culture analysis, direct microscopy and PCR. Rifampicin susceptibility testing was performed with culture-based MGIT assays and rpo sequence analysis.
Results: Out of the 878 clinical specimens, 120 were from TB-positive patients. In comparison with culture, the sensitivity of the real-time PCR test Cobas TaqMan MTB test and the Amplicor *M. tuberculosis* test were 99.0% and 95.5% for smear-positive samples and 83.0 and 72.1% for smear-negative samples, respectively. Interestingly, 15 specimens from TB-positive patients were real-time PCR positive and Amplicor PCR negative. These specimens included 8 airway samples and 7 extra-pulmonary samples. A single TaqMan MTB test positive and culture negative specimen was found. The specificity of the *M. tuberculosis* PCR tests were challenged with DNAs and cultures from strains of *Mycobacterium* ulcerans and *M. marinum,* which are the mycobacterial species most closely related to the *M. tuberculosis* complex, resulting in negative PCR test results. Less than 2% of the isolates were rifampic in resistant, reflecting the low level of *M. tuberculosis* drug resistance in Norway.
Conclusions: A comparison of two *M. tuberculosis*-specific PCR tests was performed, and the sensitivity of the real-time PCR test Cobas TaqMan MTB test was clearly superior to that of the Amplicor *M. tuberculosis* PCR test. The improved sensitivity, rapidity and less labour-intensive format of the Cobas TaqMan MTB test make this a valuable tool in routine tuberculosis diagnostics.

O234 A high-throughput method for the simultaneous detection of drug resistance and genotypic mutations in *M. tuberculosis* isolates

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Objective: To make the genetic screening of tuberculosis (TB) isolates suitable for low-income countries as well as endemic countries with high prevalence/incidence of drug-resistant TB. The emerging epidemic of drug-resistant TB calls for rapid diagnosis and early detection of drug resistance. This allows immediate and appropriate treatment of the patient and could thereby reduce the spread of multidrug-resistant (MDR) or extensively drug resistant TB (XDR-TB).
We have recently developed an MTB-specific multiplex assay, based on Multiplex Ligation-dependent Probe Amplification (MLPA). This method allows simultaneous detection of multiple dispersed DR mutations and genotype-specific mutations in the *Mycobacterium tuberculosis* (MTB) genome and has proved to be highly specific.
Method: The current read-out of MLPA is done by capillary electrophoresis, a method that is expensive and time-consuming and therefore difficult to implement and sustain in low-income countries. Recent developments in biotechnology have raised the opportunity to transfer the assay to a liquid array. Current MLPA-probes were modified to be compatible with such a system.
To cover the increasing prevalence of MDR- and XDR-TB isolates, targets revealing resistance to several important second-line drugs (e.g. fluoroquinolones) were included in the MLPA assay, as well as additional genotypic markers.
Results and Conclusion: The newly included MLPA-probes were very specific for the targeted SNPs, allowing the detection of prevalent second-line drug resistance mutations and a further delineation of MTB complex members. The genotyping abilities of the MLPA assay were additionally increased by the discovery of a new genotypic marker. There will be an explosion of SNP discovery in the next years as data from high throughput sequencing projects become available. Methods allowing informative SNPs to be rapidly detected will then become increasingly valuable.
Furthermore, we feel that the new analysis method for MLPA will specifically be of added value in low-income countries with high endemicity of DR-TB. The liquid array allows multi-parameter testing and could provide a standard platform for several diagnostic and

screening tests, which are traditionally performed by several different methods. Therefore, MLPA combined with this detection system can bring molecular typing of MTB clinical isolates closer to the patient than is currently feasible.

O235 Application of the rapid detection system for *M. tuberculosis* complex and rifampicin resistance Xpert MTB/RIF in decontaminated respiratory specimens, non-respiratory specimens and cultures

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Objectives: GeneXpert (Cepheid) is an automated real-time PCR system which is very easy to use and suitable for emergency use. The principle of the Xpert MTB/RIF test, running on GeneXpert, is to detect *M. tuberculosis* complex and mutations in the gene rpoB that cause resistance to rifampicin. This is done directly from at minimum of 500 µl of a respiratory sample. After 15 min treatment of the sample all stages of the sample preparation and PCR takes place within the instrument in 1 h30.
Some restrictions of use led us to validate this system on various types of samples.
Methods: Respiratory samples were decontaminated with NACl/NaOH and 500 µl of decontaminated product added to 1.5 ml of lysis buffer (provided with the Xpert product). For non-respiratory non-decontaminated samples (CSF, pleural liquid and biopsies) the sample volume was sometimes too low. In these cases distilled water was added up to 500 µl before addition of lysis buffer. For solid culture one colonie was resuspended in 500 µl of distilled water, for liquid culture 500 µl of medium were centrifuged prior to addition of lysis buffer. Fifteen decontaminated respiratory samples (PPD) and 24 non-pulmonary samples (PNP) were tested in the Xpert MTB/RIF assay, solid culture, Cobas TaqMan MTB test and cultures were tested with probes from GenProbe for identification.
Results: Twelve PPD of 15 were negative in Xpert MTB/RIF and the COBAS TaqMan MTB Test. Culture was also negative for 10 of these. In two samples atypical mycobacterium was isolated. 3 PPD were positive in Xpert and COBAS and showed no mutations in rpoB. The culture confirmed *M. tuberculosis* sensitive to rifampicin in these 3 cases. 20 PNP of 24 were negative by both PCR techniques and culture. Four of 24 (1 CSF, 1 pleural, 2 lymph node biopsies) were positive by both techniques and showed no mutations in the rpoB gene. The culture confirmed *M. tuberculosis* sensitive to rifampicin in these 4 cases. Nine culture isolates tested were identified as *M. tuberculosis* by Xpert and GenProbe. One isolate was determined resistant to rifampicin in Xpert. This was confirmed by susceptibility testing.
Conclusion: These preliminary results obtained on a limited number of samples show a perfect match for Xpert MTB/RIF with conventional laboratory tests irrespective of the nature of the sample tested.

O236 Evaluation of the GeneXpert® MTB/RIF assay for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis* isolates

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Introduction: *Mycobacterium tuberculosis* is one of the most significant causes of death from an infectious agent. The incidence of pulmonary tuberculosis in Turkey is nearly 27.9 per 100,000 populations. The proportion of multidrug resistant tuberculosis among new cases is 2.9% and among previously treated cases is 15.5%.
DNA sequencing studies demonstrate that 95% of rifampicin (RIF)-resistant *M. tuberculosis* strains has a mutation within the 81-bp hotspot region of the rpoB gene. The GeneXpert® MTB/RIF Assay (Cepheid, Sunnyvale, California) is a novel real-time PCR-based method for the diagnosis of tuberculosis and rapid detection of RIF-resistance in clinical specimens. However, there is a limited data about the performance of the GeneXpert® MTB/RIF Assay to detect different rpoB gene mutations.
Objectives: We aimed to determine the performance of the GeneXpert® MTB/RIF Assay for detection of the mutation within the 81-bp hotspot region of the rpoB gene in RIF-resistant *M. tuberculosis* isolates.
Methods: A total of 37 *M. tuberculosis* isolates (27 RIF-resistant, 10 RIF-susceptible), were included in the study. Drug susceptibility testing for RIF had been previously performed with the proportional method on 7H10 medium due to the criteria of the CLSI. Mutations in the rpoB gene for each isolate had also been determined by DNA sequencing.
Results: Thirteen different mutations (531-TTG, 526-CGC, 531-TGG, 526-TAC, 522-TGG, 516-TAC, 515-ATC, 533-CCG, 533-CCG, 526-TGC, 516-GTC, 513-CCA, 490-CAT, and insertion of CGG between 514 and 515) were studied in 27 RIF-resistant *M. tuberculosis* isolates (Table 1).
The GeneXpert® MTB/RIF was able to detect the mutation within the 81 bp of the rpoB gene in 26 of the 27 RIF-resistant strains. However, one isolate had 490-CAT mutation outside the 81 bp of the rpoB gene was identified as susceptible by GeneXpert® MTB/RIF. The assay also correctly identified 10 RIF-susceptible isolates.
Conclusion: GeneXpert® MTB/RIF correctly identified RIF-resistance, except one isolate which had a mutation outside the hot-spot region. In conclusion, this assay can be useful for the prediction of RIF resistance in clinical specimens and also for rapid screening of RIF resistant *M. tuberculosis* isolates.

Table 1. Comparison of GeneXpert® MTB/RIF System with DNA sequencing data of RIF-resistant isolates of *M. tuberculosis* ($n = 27$)

No. of isolates	Nucleotide change(s)	GeneXpert MTB/RIF result/ unbounded probe(s)
1	490 CAG to CAT	S*
1	513 CAA to CCA	R# (B)
1	514 515 ins. CGG	R (B)
2	516 GAC to TAC	R (B)
1	516 GAC to GTC	R (B)
2	522 TCG to TGG	R (C)
4	526 CAC to CGC	R (D)
2	526 CAC to TAC	R (D)
1	526 CAC to TGC	R (D)
7	531 TCG to TTG	R (E)
3	531 TCG to TGG	R (E)
1	533 CTG to CCG	R (E)
1	515 ATG to ATC 533 CTG to CCG	R (B, E)

*Susceptible; #Resistant.

O237 Detection by genotype MTBDRsl test of complex resistance mechanisms to second-line drugs and ethambutol in multidrug-resistant strains of *Mycobacterium tuberculosis*

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Objectives: The Genotype MTBDRsl test aims at rapid detection of resistance to ethambutol, fluoroquinolones and second line aminoglycosides (amikacin, kanamycin) and cyclic peptide (capreomycin) in *Mycobacterium tuberculosis* (Mtb).
Methods: A set of 41 MDR (multidrug-resistant) and 8 XDR (extensively drug-resistant) Mtb strains has been tested by MTBDRsl and DNA sequencing of the resistance-determining regions in gyrA and gyrB (fluoroquinolones), rpsl (streptomycin), rrs and tlyA (aminoglycosides-cyclic peptide) and embB (ethambutol).
Results: The values of sensitivity/specificity of the MTBDRsl test were as follows: fluoroquinolones 87/96%, amikacin 100/100%, kanamycin 77/100%, capreomycin 80/98% and ethambutol 57/92%. Analysis of the discrepant results indicated that 3 FQ-resistant strains (including one XDR) with mutations in gyrB were missed by MTBDRsl, and that one FQ-susceptible strain, identified as resistant by MTBDRsl, had a double

mutation T80A-A90G in GyrA not conferring resistance to FQ. Five strains (including 2 XDR) without mutation in rrs were mono-resistant to aminoglycosides or cyclic peptide and were missed by MTBDRsl. Finally, 16/28 ethambutol-resistant strains had a mutation at codon 306 in embB while 2/24 ethambutol susceptible strains had such a mutation.
Conclusions: MTBDRsl efficiently detects the most common mutations involved in resistance to fluoroquinolones, aminoglycosides-cyclic peptide and ethambutol and accurately assesses susceptibility to amikacin. However, due to mutations not included in the test (particularly in gyrB) or yet-uncharacterized resistance mechanisms (particularly those related to ethambutol and to aminoglycosides-cyclic peptide mono-resistance), the wild-type results yielded by the MTBDRsl test need to be confirmed by drug susceptibility testing when the prevalence of resistance is high.

O238 Molecular diagnosis of tuberculous meningitis – a 4-year experience

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Objectives: Tuberculous meningitis (TBM) is the most severe form of extrapulmonary tuberculosis, with high mortality rates and serious long-term consequences. Accurate and early diagnostic confirmation is essential in patient management. The purposes of this study was to, retrospectively, evaluate the performance of a molecular test for the detection of *Mycobacterium tuberculosis* complex in cerebrospinal fluid specimens (CSF), compared with culture and microscopy, and correlate the results with clinical findings.
Methods: Between November 2005 and September 2009, 118 CSF specimens were sent to the Molecular Biology Unit of Centro Hospitalar do Porto, with requests for *M. tuberculosis* molecular detection (MTD, GenProbe), with mycobacterial culture (MGITTM and Lowenstein-Jensen) and microscopy examination performed in the Microbiology laboratory. MTD test was performed as described by manufacturer, but after mechanical cell lysis, nucleic acid purification was carried out in EZ1 BioRobot (Qiagen). Presence of amplification inhibition was verified in every sample. MTB diagnosis was based in laboratory results and clinical criteria, such as patient response to anti-bacillary therapy.
Results: 118 samples from 107 patients were studied. Molecular detection of *M. tuberculosis* complex was positive in 5 cases, which were confirmed by cultural methods. Microscopy revealed acid-fast bacilli in one sample. MTB diagnosis (laboratory and clinical criteria) was established in 17 cases, 12 being presumptive. All 17 patients showed altered cytology with pleocytosis. Protein level was elevated in 16 samples (0.55–10.5 g/L). Compared with MTB diagnosis, MTD showed a sensitivity value of 30%, specificity of 100%, positive predictive value (PPV) of 100% and a negative predictive value (NPV) of 89%.
Conclusion: Our results suggest the potential role of molecular methods in confirming diagnosis of MTB, with short turnaround time results (2.5 h), high PPV, and similar sensitivity as culture (30%). Results must be interpreted in parallel with clinical findings and conventional microbiology methods. The use of molecular tests should not exclude a diagnosis of MTB (NPV-89%). More studies would help improve the adequacy of these methods.

O239 Real-time PCR for broad detection of the *Mycobacterium tuberculosis* complex and medically important atypical mycobacteria

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Objectives: To determine the sequence of the rnpB gene, coding for the ubiquitously present ribonuclease P RNA, and develop a real-time PCR for detection of the *Mycobacterium tuberculosis* complex and atypical mycobacteria.
Methods: The rnpB sequences of 17 *Mycobacterium* spp. were determined. Based on obtained rnpB sequences, two quantitative real-time PCRs for detection of the *M. tuberculosis* complex (Mytu PCR) and atypical mycobacteria (Myat PCR) were developed and combined into a single tube format. The analytical sensitivity of the PCR assay was determined with serial dilutions of target DNA. The specificity of the duplex PCR assay was tested with 21 mycobacteria species (55 strains), and 35 bacteria species other than mycobacteria. The PCR assay was evaluated on 10 samples from a quality control panel (QCMD) and on 442 clinical samples. The results were compared with the results of culture, direct microscopic examination and the Roche Amplicor PCR.
Results: Obtained rnpB sequences showed hypervariable regions enabling species specific identification and PCR design. The analytical sensitivity for detection of the *M. tuberculosis* complex was <50 copies/reaction, while for atypical mycobacteriae it was 500 copies/reaction. The assay was specific and did not detect any of 35 non-mycobacterial bacteria spp. The Mytu PCR specifically detected all four species of the *M. tuberculosis* complex and the Myat PCR detected all tested 17 atypical mycobacteria species, except 2 of 7 strains from the *M. avium* complex.
The PCR assay correctly detected all 10 samples from QCMD quality assurance panel. In analysis of 442 clinical specimens *M. tuberculosis* was detected in 40 cases (9%) by Mytu PCR, in 38 cases (8%) by the Roche PCR, in 46 cases (10%) by culture and in 23 (5%) cases by direct microscopic examination. The atypical mycobacteriae were detected by Myat PCR in 18 (4%) cases, in 11 (2%) by culture cases, and in 8 cases (2%) by direct microscopic examination.
Conclusions: Sequence determination of the rnpB gene is useful for *Mycobacterium* species identification.
Our duplex real-time PCR was shown to detect strains from both the *M. tuberculosis* complex and tested atypical mycobacteria strains. The PCR assay has a broader detection range of mycobacteria than commercial standard PCR method and has a sensitivity similar to culture.

O240 Molecular differentiation of *Mycobacterium tuberculosis* complex members from non-tuberculous mycobacteria

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Objective: Most mycobacterial infections are still caused by *Mycobacterium tuberculosis* complex (MTC) members; however, infections caused by non-tuberculous mycobacteria (NTM) are increasing, particularly among immunocompromised patients. NTM are ubiquitous in the environment and are responsible for several diseases in humans known as mycobacterioses. More than 128 mycobacterial species are currently described. Conventional species-specific identification and proper patient management are delayed due to the slow-growing nature of mycobacteria, their biochemical properties and antituberculotic drugs sensitivity testing. We propose simple molecular based method using multiplex PCR (mPCR) for rapid detection and differentiation of NTM from MTC followed by sequence analysis used for NTM identification.
Methods: In the frame of study were have analysed: 50 MTC and 57 NTM isolates collected in 2009 in the Czech Republic; 14 non mycobacterial (non myco) reference strains. Bacterial supernatant applied as DNA template for the mPCR reaction targeting rpoB gene was used for detection and differentiation of isolates as MTC or NTM. One amplicon of 235 bp from MTC members or a single amplicon of 136 bp from NTM are expected. NTM identification was archieved by DNA sequence analysis of the species-specific 16S rRNA gene region.
Results: The mPCR reaction with template DNA from mycobacteria yielded the expected amplicons. We assume that proposed mPCR is specific for mycobacteria because analysis of non myco strains resulted in any amplicons. Detection limit for proposed mPCR was 50 copies per reaction, which faciliated its use for analysis of DNA extracted directly from clinical samples (sputa or biopsies). DNA sequencing of the 16S rRNA gene confirmed the status of analysed bacterial isolates and enabled precise isolate identification to the species level. Different NTM (Table 1) causing mycobacterial infections in humans were detected using this approach.
Conclusion: The mPRC used in this study allowed rapid detection and differentiation of primary cultures as MTC, NTM or non myco. Specific NTM identification is possible in second step by sequence

analysis of 16S rRNA gene, thus helping in timely institution of specific mycobacterial therapy.

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Table 1. Bacterial classification by multiplex PCR

Bacterial isolates[1]	No. of isolates	Source[2]	MTC[3]	NTM[3]	Non myco[3]
MTC	50	human	50	–	–
Mycobacterium arupense	2	human	–	2	–
M. avium subsp. *avium*	21	human	–	21	–
M. a. subsp. *hominissuis*	2	human	–	2	–
M. a. subsp. *paratuberculosis*	3	environment	–	3	–
M. celatum	1	environment	–	1	–
M. chelonae	1	human	–	1	–
M. chitae	1	environment	–	1	–
M. engboekii	3	environment	–	3	–
M. gordonae	5	environment	–	5	–
M. interjectum	2	environment	–	1	–
M. intracellulare	1	human	–	1	–
M. marinum	3	human	–	3	–
M. nonchromogenicum	3	human	–	3	–
M. peregrinum	6	environment	–	6	–
M. raccae	1	environment		1	–
M. terrae	1	human	–	1	–
M. xenopii	1	human	–	1	–
Enterobacter cloacae	1	reference	–	–	1
Enterococcus faecalis	1	reference	–	–	1
Enterococcus faecium	1	reference	–	–	1
Escherichia coli	1	reference	–	–	1
Klebsiella pneumoniae	1	reference	–	–	1
Pasteurella multocida	1	reference	–	–	1
Staphylococcus aureus	1	reference	–	–	1
Staphylococcus epidermidis	1	reference	–	–	1
Staphylococcus haemolyticus	1	reference	–	–	1
Staphylococcus warneri	1	reference	–	–	1
Salmonella spp.	1	reference	–	–	1
Streptococcus anginosus	1	reference	–	–	1
Streptococcus pasteria	1	reference	–	–	1
Streptococcus dysagalactiae	1	reference	–	–	1
Total	121		50	57	14

MTC: *M. tuberculosis* complex; NTM: non tuberculous mycobacteria; non myco: bacteria other than mycobacteria.
[1]Isolates differentiation confirmed by sequence analysis. [2]Clinical isolates obtained by cultivation from human sputum or skin biopsies; environmental isolates were obtained from water, soil, dust, biofilm, organic remains and animal samples. [3]Isolates classification obtained from multiplex PCP.

O241 Surveillance of antibiotic resistance in leprosy by a new molecular test, the GenoType® LepraeDR

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Objectives: Although leprosy is treated by multidrug therapy since 1982, resistance to the first-line drug (dapsone, rifampicin) is described for 30 years and resistance to second-line drug as fluoroquinolones is described since 1997. Since *Mycobacterium leprae* is not growing *in vitro*, its susceptibility testing (one year experiment in the mouse footpad) is rarely done. Molecular methods are thus necessary to detect antibiotic resistance in leprosy and are so far in house methods requiring sequencing. Our aim was to provide a commercially based molecular test able to detect for antibiotic resistance in leprosy.
Methods: *M. leprae* strains contained in biopsy skin samples were studied for resistance to dapsone, rifampicin and ofloxacin by the reference drug susceptibility method (the mouse footpad method). DNA was extracted and regions involved in resistance to the three drugs (rpoB gene for rifampicin, gyrA gene for ofloxacin and folP1 gene for dapsone resistance) were amplified and sequenced. The new DNA strip test, GenoType® LepraeDR, was applied blindly. Results were compared to those obtained by the genotypic method (PCR sequencing) and the phenotypic reference method (mouse footpad).
Results: Among the 91 *M. leprae* strains, 14 were resistant to rifampicin, 18 were resistant to dapsone and 1 was resistant to ofloxacin. For the resistant isolates, PCR sequencing was 100% concordant with the mouse footpad test with mutation (numbering system of the *M. leprae* genome) in rpoB (10 S456L, 2 S456M, 1 Q438V, 1 G432S), in folP1 (5 P55L, 3 P55R, 7 T53I, 2 T53A, 1 T53V) and gyrA (1 A90V). Results obtained by the GenoType® LepraeDR test were 100% concordant with the two reference methods. For the susceptible strains, there were two discordances between the mouse footpad testing and the PCR sequencing: PCR-sequencing showed in one strain a silent mutation at codon 427 in rpoB and in a second strain a serine to cysteine substitution at the same codon, which were both not associated to rifampicin resistance. GenoType® Leprae DR showed a wild-type pattern for rpoB for these two isolates.
Conclusion: Results obtained by the GenoType LepraeDR test were 100% concordant with the reference phenotypic susceptibility testing and 98% with the genotype determined by PCR and sequencing. The test is easy to perform and may be affordable in all laboratories, including those in endemic countries.

O242 A novel real-time PCR assay for specific detection and quantification of *Mycobacterium avium* ssp. *paratuberculosis* in milk with the inherent possibility of differentiation between viable and dead cells

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Objectives: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is known as the etiological agent of paratuberculosis (Johne's disease) in ruminants. Crohn's disease is an inflammatory gastrointestinal tract disease in humans, presenting with similar symptoms and pathological changes in the gut as Johne's disease in cattle. Therefore, it was suggested that MAP could be one of the etiologic factors of the disease. The aim of the present study was to develop a MAP-specific real-time PCR assay providing the additional possibility of detecting viable MAP.
Methods: A real-time PCR assay based on amplification of the specific Mptb52.16 target was designed including an internal amplification control to identify false negative results. The detection limit was established in artificially contaminated raw milk samples and the optimized assay applied to 96 naturally contaminated raw milk samples. The potential of the real-time PCR assay to detect viable MAP was explored by assessing expression of the Mptb52.16 target in raw milk samples and inoculated Dubos broth.
Results and Conclusions: The method showed 100% inclusivity and exclusivity when testing 11 MAP strains, 22 non-MAP mycobacteria, and 16 raw milk microflora strains. The detection limit in artificially contaminated raw milk was 2.42×10^1 MAP cells/ml milk. In a survey of naturally contaminated samples obtained from dairy herds with a known history of paratuberculosis, 47.8% pre-milk and 51.9% main milk samples tested positive. Real-time PCR-derived MAP-specific bacterial cell equivalents (BCE) ranged from 1×10^0 to 5.1×10^2 BCE/51.44 ml; the majority of samples had less than one BCE per ml milk. Expression of the chosen target was detected in artificially contaminated raw milk as well as inoculated Dubos broth, thus confirming the real-time PCR assay's potential to detect viable MAP cells.

Infection in the transplant recipient

O243 Acquisition of cytomegalovirus specific immune response during pre-emptive therapy protects high-risk solid organ transplant patients from infection

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Objectives: The objective of this study is to determine whether the activation of a cytomegalovirus (CMV)-specific T cell-mediated response

during pre-emptive therapy controls further replication episodes without valganciclovir administration in solid organ transplant (SOT) patients at high risk for CMV infection.
Methods: SOT recipients at high risk for CMV infection, those that are seronegative and receive a seropositive graft, were followed for 18 months after transplantation. CMV viral loads (VL) were determined by real-time PCR, and the CMV-specific immune response was characterized by flow cytometry by the detection of CD4+, CD8+ and CD3+ T cells expressing CD69 and secreting IFN-g and IL-2. Preemptive treatment was administered when VL reached 1,000 copies/ml. Once a CMV-specific immune response was detected no treatment was administered and patients were closely monitored.
Results: Eleven patients with a median age of 53 years were included. Between 2 and 7 weeks after transplantation, all patients experienced CMV replication episodes that ranged from 787 to 1,432,217 copies/ml. All of them were resolved after administration of valganciclovir. Between weeks 10 and 20 post-transplantation a CMV-specific immune response was detected in all patients. After this, 33 replication episodes were detected, 32 of which (97%) were controled by the host immune system without the administration of treatment (VL ranging from 10 to 31,317 copies/ml). Furthermore, although the number of positive PCR results was similar before and after the acquisition of immunity, VL levels were significantly lower ($p = 0.017$) after immunity than before with median values of 1,696 copies/ml and 20,110 copies/ml, respectively. From week 39 to week 51 all replication episodes were 1,000 copies/ml and no new replication episodes were detected after week 51.
Analysis of the immune response demonstrated that IL-2 was secreted from CD4+ T cells significantly earlier than IFN-g (6 vs 8.5 weeks post-transplant; $p = 0.001$). Aditionally, IFN-g was secreted from CD4+ T cells significantly earlier than from CD8+ T cells (9 vs 12 weeks post-transplant; $p = 0.005$).
Conclusion: Pre-emptive therapy promotes the acquisition of an early immune response after transplantation in SOT patients at high risk for CMV infection. We demonstrate that the immune response elicited during pre-emptive therapy confers immunity to later CMV replication events.

O244 Break-through HHV-6B infections during antiviral prophylaxis after liver transplantation

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Objectives: Human herpesvirus-6 (HHV-6) activation, mostly of the variant B, is common after liver transplantation. Most HHV-6 reactivations are asymptomatic, but symptoms such as encephalitis, hepatitis or graft dysfunction have been described. The clinical experience on antiviral therapy is very limited, but based on *in vitro* studies, the current antiviral drugs effective against cytomegalovirus (CMV), have also activity against HHV-6. However, ganciclovir is less effective against HHV-6B than HHV-6A. The aim of our study was to investigate the efficiency of antiviral prophylaxis, given to the CMV-seronegative risk patients receiving the graft from the seropositive donor, in preventing HHV-6B reactivation.
Methods: Of 232 consecutive adult liver transplant patients 36 belonging to the CMV high risk group received valganciclovir (or ganciclovir) prophylaxis up to 3 months after transplantation. The patients were frequently monitored for CMV by real-time quantitative PCR and HHV-6 reactivations were demonstrated by the antigenemia test in PBMC by using indirect immunoperoxidase staining and monoclonal antibodies against HHV-6B and HHV-6A. Intragraft HHV-6 infection was demonstrated in liver biopsies by using the same antibodies and immunostaining.
Results: During antiviral prophylaxis, no break-through CMV infections were recorded. On the contrary, HHV-6 antigenemia was detected in 13/36 (36%) patients appearing mean 12 days (range 7–22 days) after transplantation. All reactivations were caused by HHV-6B. In three cases HHV-6 antigens were also detected in the transplant associated with graft dysfunction.
Conclusions: HHV-6B reactivations were common during antiviral prophylaxis after liver transplantation. At least in three cases also the transplant was infected. Valganciclovir/ganciclovir prophylaxis did not prevent HHV-6B infections in adult liver transplant patients.

O245 Diagnosis of polyomavirus-associated nephropathy in renal-allograft recipients by real-time polymerase chain reaction and urine cytology

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Objective: Polyomavirus-associated allograft nephropathy (PVAN) frequently results in allograft dysfunction in renal transplant patients in the era of powerful immunosuppressive agents. BK virus (BKV) is the dominant causal agent whilst JC virus (JCV) accounts for the rest. Although renal biopsy is considered as the diagnostic standard for PVAN, a less invasive surrogate method is helpful. This study evaluated the clinical utility of urinary decoy cells and urine and plasma BKV/JCV real-time polymerase chain reaction (PCR) in the diagnosis of PVAN in renal transplant recipients with allograft dysfunction.
Methods: In a retrospective analysis, 22 renal transplant patients with allograft dysfunction and with renal biopsy results were evaluated. All patients received real-time PCR assays for BKV and JCV for their plasma and urine samples within one week of renal biopsy. The biopsy results included 7 renal-allograft recipients with pathological diagnosis of PVAN (5 BKV- and 2 JCV-associated) and 15 patients with pathological diagnosis other than PVAN. Urine cytology was evaluated for decoy cells within 1 week of renal biopsy in all patients.
Results: In the 7 PVAN patients, exclusive BKV viruria was detected in all patients with BKV-associated PVAN and exclusive JCV viruria was detected in 2 patients with JCV-associated PVAN. BKV viremia exceeded the cut-off threshold value (10^4 copies/ml) was found in all 5 BKV-associated PVAN patients. JCV viremia was found in one JCV-associated PVAN patient. The use of decoy cells as a marker of PVAN had a sensitivity of 85.7% and negative predictive value of 93%. The use of polyomavirus viruria in aiding the diagnosis of PVAN had a sensitivity of 100% and negative predictive value of 100%. The use of polyomavirus viremia in aiding the diagnosis of PVAN had a specificity of 93.3% and positive predictive value of 85.7%.
Conclusions: BKV and JCV real-time PCR methods may help in the diagnosis of PVAN among renal transplant patients. BKV viremia ($>10^4$ copies/ml) is highly associated with BKV nephropathy whilst the cut-off value for JCV requires further investigation.

O246 Norovirus-related severe and chronic diarrhoea in renal transplant adult recipients

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Objectives: Noroviruses (NoV) and Sapoviruses (SpV) are two separate genera in the Caliciviridae family. NoV are an increasingly recognized cause of transient gastroenteritis in immunocompetent individuals and have been recently implicated in chronic diarrhea in immunocompromised patients. However, clinical and epidemiological spectrum of Caliciviridae infections in renal transplant recipients is still poorly defined.
Methods: We undertook a single-center retrospective analysis of the clinical and virologic features of Caliciviridae infection, diagnosed by reverse transcription polymerase chain reaction (RT-PCR) from July 2008 to September 2009. Genotypes and variants were identified by genome sequencing. Other infectious agents were ruled out.
Results: Eleven renal transplant adult recipients were identified as having Caliciviridae infection, including 10 NoV and 1 SpV. All of them were given an induction immunosuppressive therapy relayed by a triple maintenance therapy. The median time from transplantation to symptoms and from then to diagnosis were 21 months (range: 1–83) and 30 days (range: 9–154), respectively. All patients had protracted

diarrhea, requiring hospitalization and IV rehydration. Vomiting and fever were present in 5 (45%) and 2 (18%) patients, respectively. All but one experienced weight loss with an average loss of 4.5±2.2 kg, 8 (72%) of them presented with acute renal failure. Patient-to-patient nosocomial transmission was very likely in two patients who shared the same hospital room and in whom the same variant was identified. All others had community-acquired-norovirus infection and one in the context of a family outbreak. NoV genotypes found were GI-3, GII-2, GII-4, GII-6 variants in 1, 1, 6 and 2 patients, respectively. Diarrhea was lasting more than 4 weeks in 10 of them with a median duration of 56 days (range: 8–261). Strikingly, diarrhea resolved after mycophenolate tapering or withdrawal in 5 (46%) and 4 (36%) patients, respectively. All patients survived and none experienced a relapse of diarrhea with a median follow-up of 5 months (range: 1–17).
Conclusion: NoV is a likely underestimated cause of severe and chronic diarrhea in renal transplant recipients. This may delay effective therapeutic intervention that mostly consists in reducing immunosuppression. Prospective studies are warranted to assess the correlation between immunosuppressive regimens, resolution of diarrhea and virus clearance.

O247 Evolution of anelloviridae strains in serial blood and biopsy samples from a renal transplant patient

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Objectives: The Anelloviridae family is composed of multiple viral species belonging to the prototype TT virus. Biology and implications for host health of this intriguing viral family is still a matter to debate. In order to better understand the evolution of anelloviridae strains in the immunocompromised host, we performed a molecular analysis of serial blood and biopsy samples from a kidney transplant recipient.
Methods: Viral DNA was extracted from plasma samples using the High pure viral nucleic acid kit (Roche). Biopsy tissue samples were pre-digested using proteinase K, and nucleic acids were further extracted using the same approach. Rolling circle amplification (RCA) using Phi29 DNA polymerase was performed on extracted materials in order to optimise the detection of viral DNA. Anelloviridae detection was performed using a highly conserved PCR system; amplified products were further cloned and sequenced, and submitted to phylogenetic analyses.
Results: The evolution of viral strains detected in blood samples was bimodal: 1) prior to kidney transplant (T-90 days to T0), the genetic diversity exhibited by anelloviridae sequences was moderate; 2) on the other hand, multiple variants were detected from T+15 to T+420 (end of follow-up).
Regarding the biopsy samples, although the first sample (T+11) was PCR negative, an anelloviridae sequence was identified in the second sample (T+170). Interestingly, this sequence was identical or nearly identical to a cluster of sequences identified in the blood of the patient before transplant.
Conclusion: This study suggests that a transplant is able to be colonized by an anelloviridae strain identified previously in the blood of the corresponding patient. It confirms also that major changes in anelloviridae dynamics occur in the blood of patients undergoing immunosuppressive treatments. Precise analysis of biological and molecular data collected in this study is exposed.

O248 Relationship of viral load Epstein–Barr virus as a marker predictive lymphoproliferative disease after liver transplant

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Background: Lymphoproliferative disease (ELPT) are a heterogeneous group of lymphoid disorders that can develop in patients undergoing solid organ transplantation. The incidence ranges between 2% adults and 10% in children and reach a mortality rate between 40 and 70%. The wide range of clinical presentations ranging from a mononucleosis syndrome and lymphadenopathy, masses to multiple organ dysfunction. The risks identified in pediatric patients post transplant are the early ages, the use of Tacrolimus, a high viral load and a concomitant low cellular immune response.
Objectives: To describe the relationship between EBV viral load as a predictive marker for the presence of ELPT and clinical characteristics of patients after transplantation of liver in the Hospital Infantil de Mexico "Federico Gomez" in the period January 2005 to January 2008.
Methods: A descriptive, retrospective and observational study of a series of cases.
Results: From January 2005 to January 2008, nine liver transplants were performed, three were high risk by serology (EBV D-/ R +), of which one ELPT development at 100 weeks post-transplant viral load of 1450 genomes / mL plasma. The time of reactivation of EBV infection on average for the nine patients was 3.3 weeks after transplantation. Seven patients belonged to age group <5 years (high risk by age). And 4 ELPT developed ELPT viral loads >800 genomes/mL of plasma. Five of nine patients developed ELPT diagnosed by liver biopsy and immunohistochemistry. All patients had elevated liver enzymes more than 4 times its baseline, 2 patients had atypical lymphocytes in the time of diagnosis and 3 ELPT monocytosis. Two patients had clinical mononucleosis-like and 3 were asymptomatic at the time of diagnosis of ELPT with viral loads of >900 genomes/mL. The patient number 9 ELPT present two events with added diagnosis of acute cellular rejection by biopsy in his second event of ELPT and high viral loads.
Conclusions: Viral load >800 genomes/mL accompanied by elevation in liver enzyme values of up to four or more times their normal levels were related to the development of ELPT.

O249 Reduced IgG antibody avidity in organ transplant recipients after varicella-zoster-virus vaccination

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Objectives: Varicella-zoster virus (VZV) infection may cause significant morbidity and mortality in the immuno-compromised patient. VZV vaccine induces both humoral and cellular immunity. However, there is no clear correlation between IgG antibody titers and protection against infection. Antibody protection is a function of both concentration and affinity (=chemical binding strength). Avidity is a measure of functional affinity. It has been suggested that studies of vaccine efficacy should incorporate analysis of avidity, since high avidity is a marker of memory priming. The present study was designed in order to answer the question as to whether solid organ transplant (SOT) recipients have a lower VZV IgG antibody avidity, despite having protective IgG antibody levels after vaccination, or not.
Methods: The serum samples of SOT recipients were evaluated for IgG antibody levels against VZV and IgG antibody avidity. Twenty-eight patients were included in the study (20 had had liver transplantation, 3 heart transplantation, 5 kidney transplantation) and had received a single dose of Varivax (Aventis Pasteur, Lyon, France) prior to transplantation. The control group consisted of 50 healthy children, 36 of whom had had clinical and serological confirmed varicella infection after wild-virus contact and 14 of whom had varicella vaccination with a single dose of Varivax.
Results: Median IgG antibody levels were 800 U/ml in wild-virus infected controls, 810 U/ml in vaccinated controls and 630 U/ml in SOT recipients. Median relative avidity index (RAI) was 89% for wild-virus infected controls, 94% for vaccinated controls and 82% for transplant recipients ($p = 0.01$ compared to wild-virus infected controls, $p = 0.002$ compared to vaccinated controls).
Conclusions: In conclusion, IgG antibody avidity in SOT recipients may serve as a substitute marker to evaluate humoral immunity against VZV. This is of particular importance in the clinical setting of exposure to VZV when considering VZV-specific immunglobulin and/or acyclovir treatment in order to prevent clinical relevant re-infection and varicella-caused complications as a result of exposure to VZV after transplantation. However, the role of humoral protection against VZV has to be evaluated in long-term follow-up, since also cellular immunity may play a crucial role in defence against viral infections.

O250 Characteristics and timing of bloodstream infections following orthotopic liver transplantation: a single-centre experience

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Aims: To analyse the characteristics of bloodstream infections (BSI) occurring in orthotopic liver transplant (OLT) recipients in an Irish tertiary care hospital during the 2 year period from July 1st 2007 to June 30th 2009. To compare the findings with data from a previous time period.
Methods: The incidence, timing, aetiology and microbiology of BSIs in OLT recipients were obtained retrospectively. 124 OLTs were performed from July 1st 2007 to June 30th 2009. Chart reviews were performed on all patients who had positive blood cultures up to 12 weeks post-OLT. Bacteraemia or fungaemia was considered to be significant according to the CDC criteria. Previous related literature was reviewed.
Results: Overall 21 BSIs were recorded with an incidence of 17 BSIs per 100 patients transplanted. 75% of all BSIs occurred within the first two weeks post-OLT. The most frequent sources of BSI were abdominal (52%) and intravascular catheters (32%). Gram-positive organisms were the most frequent aetiological agents overall (43%). Gram-negative organisms were the primary aetiological agents in the early post-operative period (<1 week). In week one post-OLT, 73% (8/11) of BSIs were due to Gram-negative organisms (85% abdominal source). Gram-positive organisms predominated after the first post-operative week, causing 70% (7/10) of BSIs. Crude mortality of BSIs was 33%, being highest in the early post-operative period. Among the Gram-positive isolates, 8% were vancomycin-resistant enterococci and there was no methicillin-resistant *Staphylococcus aureus* isolated. Among the Gram-negative isolates, 70% were ESBLs and 30% were AmpC producers. Overall the incidence of BSI at our transplant centre has decreased between the two time periods. In the period 1995 to 2000, 91 OLTs were performed with an incidence of 28 BSIs per 100 patients transplanted. Gram-positive organisms were the most frequent causative agents during this period (60%).
Conclusion: BSIs remain a major concern in OLT recipients. An understanding of the aetiology of BSI post-OLT is important for empiric antibiotic choice in the event of infectious complications post-surgery. In an attempt to further reduce the incidence of BSI post-OLT we plan to target IV catheter-related sepsis at our centre.

O251 Multi-resistant pathogens – a problem in nosocomial bloodstream infections in patients with haematopoetic cell transplantation? Evaluation of ONKO-KISS data

D. Luft, R. Babikir, H. Bertz, A.F. Widmer, W. Kern, M. Dettenkofer and the ONKO-KISS Study Group*

Objectives: To assess the relevance of three multiresistant pathogens (methicillin-resistant *Staphylococcus aureus* [MRSA], extended-spectrum β-lactamase [ESBL] producing *Escherichia coli*, vancomycin-resistant [VR] *Enterococcus* facium) in nosocomial bloodstream infection (BSI) during neutropenia in adult patients undergoing hematopoetic cell transplantation (HCT), data derived from the ongoing multicenter surveillance project ONKO-KISS were evaluated. The project was initiated by the German National Reference Centre for Surveillance of Nosocomial Infections in 2000.
Methods: Nosocomial bloodstream infections are identified using CDC definitions for laboratory-confirmed BSI [for detailed information see: CID 2005; 40: 926, or in German language: http://www.nrz-hygiene.de/surveillance/onko.htm]. Data on BSI and pathogens detected in blood cultures as well as key resistance patterns are reported to the Reference Centre for Surveillance.
Results: From January 2004 up to June 2009 24 centers participated. Altogether 6,303 patients with 93,026 neutropenic days were investigated. 984 cases of nosocomial BSI were diagnosed and a total of 1101 pathogens were detected, of whom 37 belonged to the above mentioned multiresistant pathogens (3.4%). 7 out of 22 *S. aureus* isolates were MRSA (31.8%), 17 out of 174 *E. coli* isolates were ESBL-producers (9.8%) and 13 out of 63 *E. faecium* isolates showed Vancomycin resistance (20.6%). For details see Table.
Conclusion: The overall number of BSI involving multiresistant pathogens in the ONKO-KISS patient group is rather low. Rates of MRSA and VR *E. faecium* were varying in the observation period and sometimes higher than the corresponding rates for Germany derived from EARSS [European Antimicrobial Resistance Surveillance System; www.rivm.nl/earss]. However, the low overall numbers must be taken into account. ESBL producing *E. coli* were not seen before 2007 but thereafter with a high percentage in comparison to EARSS data for 3rd generation cephalosporin resistant *E. coli*.

Year	MRSA/*S. aureus* total no. (%)	ESBL producing *E. coli*/*E. coli* total no. (%)	Vancomycin resistant *E. faecium*/*E. faecium* total no. (%)	Total no. of blood culture isolates
2004	2/7 (29%)	0/27 (0%)	2/6 (33%)	167
2005	0/3 (0%)	0/34 (0%)	8/18 (44%)	220
2006	3/5 (60%)	0/25 (0%)	2/9 (22%)	199
2007	1/4 (25%)	5/38 (13%)	0/9 (0%)	206
2008	0/2 (0%)	10/37 (27%)	1/18 (6%)	230
01–06 2009	1/1 (100%)	2/13 (15%)	0/3 (0%)	79
Total	7/22 (32%)	17/174 (10%)	13/63 (21%)	1101

O252 Quantification of CMV DNA in plasma by real-time PCR for management of allogenic stem cell transplant recipients

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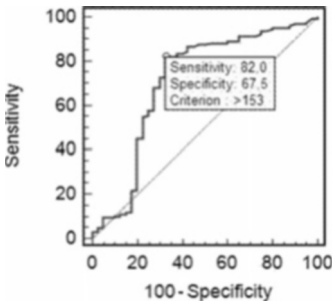
The objective was to evaluate a quantitative real time PCR in plasma samples for monitoring active CMV infection in allogenic stem cell transplant (allo-SCT) patients and try a new strategy for the initiation of CMV preemptive therapy.
We studied 395 plasma samples obtained of 51 CMV infection episodes from 32 allo SCT patients between January 2007 and February 2009. 29 out of 32 received one or more course of preemptive therapy upon positive AG and/or TNAI-PCR results and 6 (19%) developed CMV end-organ disease (4 colitis and 2 neumonitis). All patients were monitored post-STC with antigenemia pp65 CINApool®, Argene (AG) and conventional quantitative PCR, COBAS® Amplicor® CMV after automatic extraction COBAS® Ampliprep® TNAI kit, Roche (TNAI-PCR). A positive sample was defined by AG $\geqslant 2/4\times10^5$ PMNs and/or TNAI-PCR $\geqslant$600 copies/mL.All samples were retrospectively tested using a real time PCR Affigene® CMV trender after automatic DNA extraction with NucliSENS® easyMAG®, bioMérieux (rt-PCR); data lower than 57 copies/ml were considered as negative. An episode was defined as the period between the first positive sample by antigenemia and/or TNAI-PCR, until the first negative sample by both techniques. Plasma samples were positive in 28.3%, 30.6% and 46% by AG, TNAI-PCR and rt-PCR, respectively. Rt-PCR detected 72% of AG positive samples vs 60% by TNAI-PCR. AG was not evaluable in 30 samples (7.5%). Sixteen samples (53%) were positive by rt-PCR vs 7 (23%) by TNAI-PCR. 12% of samples were only positive by rt-PCR. The range for rt-PCR was 63–2.77×10^4 copies/ml vs 608–2.7×10^4 copies/ml for TNAI-PCR. Episodes were detected in 46 (90%), 38 (82%) and 41 (89%) by AG, TNAI-PCR and rt-PCR, respectively. Six episodes (11.8%) were only detected by AG (all PCRs negative). Five episodes (10.9%) were detected only by PCR assays. Thirty-four episodes out of 51 episodes (66.7%) were detected by both PCR techniques, in 14 out of 34 (41.2%) were detected earlier by RT-PCR than TNAI-PCR (median = 7 days), and in 5 (14.7%) TNAI-PCR was earlier than Rt-PCR (median = 3 days). Concordance between PCRs assays was 79% (k = 0.6). Strategy based on the quantification of CMV DNA in plasma could be established in >153 copies/Ml (curve ROC, graphic 1) for triggering the initiation of CMV preemptive therapy in this clinical setting. Rt-PCR can be introduced to allow the more sensitive, rapid, and accurate diagnosis of CMV reactivation infection in SCT recipients, which allowed for preemptive therapy to be administered as early as possible.



Graphic 1. EASYMAG.

Treatment of hospital-acquired candidaemia: strategies, opinions and practice (Symposium supported by Astellas)

S253 Treatment of hospital-acquired candidemia: strategies, opinions and practice. A question-and-answer expert workshop

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Invasive *Candida* infections occur in many different patient populations. Critical care is widely acknowledged as an important predisposing factor, with a third of candidaemia episodes occurring in the ICU. This is chiefly because ICU patients are exposed to many of the risk factors, which include compromises to natural barriers of infection; *Candida* colonisation and alterations in normal flora; and the presence of debilitating diseases or immunosuppression, particularly neutropenia. Invasive *Candida* infections also occur outside the ICU, as the cardinal risk factors are present in a variety of patient types, including patients undergoing major abdominal surgery; bone marrow and solid-organ transplant patients; and patients undergoing cancer chemotherapy.

Although risk factors for invasive *Candida* infections are well characterised, definitive diagnosis remains problematic due to the long lead times associated with blood culture and histopathology. Targeted treatment, i.e. selection of an antifungal agent tailored to a particular *Candida* species with known antifungal susceptibility, is rarely possible initially. Consequently, pre-emptive and empirical strategies, which make use of the limited information available to guide treatment decisions, are commonly employed.

Patients with invasive *Candida* infections are often severely ill prior to developing a fungal infection. Therefore, rapid initiation of therapy is vital, as the risk of mortality increases significantly with any delay in treatment. However, it is also important to consider carefully the choice of agent, as differences exist in microbiological and clinical efficacy. Many cases of invasive *Candida* infections are caused by species that are potentially resistant to older antifungals, particularly fluconazole. Therefore, if the causative agent is unknown, or is suspected to be a fluconazole-resistant species (i.e. *C. glabrata* or *C. krusei)*, a broad-spectrum antifungal agent should be selected first-line, with recent guidelines recommending echinocandins as the best overall choice.

Differences also exist between the echinocandins, particularly with regard to their suitability for use in certain patient populations. Therefore, careful assessment of individual cases is the optimal basis for selection of therapy. Micafungin, which was evaluated in an extensive clinical trial programme and has to date been administered in 600,000 patients worldwide, is a recognised treatment option.

Blood, bone and biofilm: improving outcomes in systemic and deep-seated infections (Symposium supported by Novartis)

S254 Management of catheter-related bacteraemia

P. Grossi (Varese, IT)*

Intravascular catheters are indispensable in current medical practice. However, they provide surfaces for microbial colonisation and biofilm formation and predispose patients to bacteraemia. Some of the most common causative pathogens, such as coagulase-negative staphylococci and *Staphylococcus aureus*, have adherence properties that are important in the pathogenesis of catheter-related infections. The prevalence of multidrug-resistant pathogens, the need for rapidly effective empiric therapy to avoid complications and improve outcomes and the altered pharmacokinetics of septic patients make bacteraemia a significant clinical challenge.

The Infectious Diseases Society of America (IDSA) recently published updated guidelines for the management of catheter-related bacteraemia. Catheter removal and prompt systemic antibiotic therapy are central to both complicated and uncomplicated bacteraemia management. The IDSA and other guidelines highlight ongoing clinical challenges, which more recent antibiotics are helping to address. For example, clinical evidence of reduced MRSA susceptibility to vancomycin has prompted the IDSA to recommend daptomycin* in institutions where vancomycin MICs >2 μg/ml are common. Bacteraemia caused by vancomycin-resistant enterococci is also of increasing concern. Therapeutic options are limited and recent novel antibiotics may be helpful in these cases.

Haemodialysis patients have particular risk factors for catheter-associated bacteraemia. Catheter removal may pose logistical problems; however, failure or delay in removal increases the risk of haematogenous spread. Compared with catheter retention, removal of *S. aureus*-infected catheters is associated with a more rapid response to therapy and/or a higher cure rate. Furthermore, success rates for catheter salvage using adjunctive antibiotic lock therapy are lower for Gram-positive pathogens, such as *S. aureus*, enterococci and *Staphylococcus epidermidis*, than for Gram-negative infections. The IDSA provides dosage recommendations for daptomycin and linezolid for dialysis patients.

This presentation will discuss these and other topical clinical challenges in the management of catheter-related bacteraemia.

*Daptomycin is licensed in Europe for the treatment of complicated skin and soft tissue infection (cSSTI), for *S. aureus* right-sided infective endocarditis (RIE) and for *S. aureus* bacteraemia when associated with cSSTI or RIE.

S255 Management of non-catheter-related bacteraemia

J.M. Miró (Barcelona, ES)*

Patients with Gram-positive non-catheter-related bacteraemia are at high risk of septic metastases, particularly in bones and joints (especially if prostheses are present), the epidural space, intervertebral discs and cardiac valves. The presentation of non-catheter-related bacteraemia (which includes primary bloodstream infection [BSI] and bacteraemia secondary to infections in other sites acquired either in the community or in nosocomial settings) is heterogeneous and may require diagnosis and treatment of infections in foci beyond the blood.

A dominant pathogen in both nosocomial and community-acquired bacteraemia is *Staphylococcus aureus*, and *S. aureus* bacteraemia is associated with a high frequency of complications, including infective endocarditis (IE). Mortality is significantly worse in patients with primary or secondary BSI compared with catheter-related bacteraemia and also in those infected with MRSA vs MSSA. Furthermore, inappropriate empiric treatment for patients with Gram-positive bacteraemia increases mortality risk.

Treatment guidelines for bacteraemia are primarily based on specific infection foci, such as IE and catheter-related infections. Empiric

treatment for bacteraemia should take account of the likely pathogen and local susceptibility patterns, in addition to presenting symptoms, patients' general heath status and medical history (including risk factors for MDR involvement), comorbidities (e.g. renal impairment) and concomitant medications. When the infection focus is not a vascular catheter, management should also address the risk of complications, particularly related to *S. aureus*, and the need for some patients to receive prolonged therapy, potentially with initial hospital treatment followed by continued antimicrobial therapy post-discharge.
Daptomycin* is one of the few antibiotics with efficacy and safety in the treatment of *S. aureus* bacteraemia, including that associated with IE. Rapid bactericidal activity, including against biofilms and stationary phase bacteria, and the potential for outpatient treatment with the once-daily 2-minute intravenous injection, are further characteristics of daptomycin that may be useful for non-catheter-related bacteraemia.
*Daptomycin is licensed in Europe for the treatment of complicated skin and soft tissue infection (cSSTI), for *S. aureus* right-sided infective endocarditis (RIE) and for *S. aureus* bacteraemia when associated with cSSTI or RIE.

S256 Continuous challenges in hospital-acquired deep-seated infections: need for antibiotics with activity against biofilm

A. Trampuz (Lausanne, CH)*

Prosthetic joint infection (PJI) and osteomyelitis (OST) are difficult to eradicate and remain an ongoing challenge for treating physicians. Until recently, prosthetic surgery was usually approached as either a two-stage exchange, separated by a long interval (6–8 weeks), or life-long antibiotic suppression therapy. The first approach is associated with prolonged hospitalisation and risks of secondary complications; the second has a low probability of success. Antimicrobials effective against slow-growing, stationary growth-phase and adherent microorganisms are needed to eradicate biofilm infections.
This presentation will discuss novel treatment concepts and their impact on long-term outcomes and also recently updated treatment guidelines for PJI and OST. The choice to retain or remove an implant depends on the patient profile: in specific groups, implant infections can be cured by early debridement and long-term treatment with antibiotics that have good biofilm activity. Antimicrobial type, doses and duration of therapy will be discussed. Potentially, newer antibiotics such as daptomycin* may prove to be valuable options in implant-associated methicillin-resistant staphylococcal and enterococcal infections. Treatment protocols of phase II studies with daptomycin with or without rifampin in PJI and OST will be reviewed as well as the use of higher doses of daptomycin. Common pitfalls and reasons for treatment failure will be outlined. Empiric antibiotic therapy should not be administered before microbiological diagnostic confirmation, which is difficult when antibiotics are administered for a draining wound overlying an implant. In addition, the risk of development of antimicrobial resistance is high, particularly for rifampin. Therefore, rifampin should be avoided if there is contact between the body surface and the implant (e.g. open wounds, wound dehiscence, fistulae, vacuum-assisted closure foam or drainage). Other common mistakes are insufficient duration and/or dose of antibiotics and switching from intravenous to oral agents with insufficient bioavailability or inactivity against biofilms.
*Daptomycin is licensed in Europe for the treatment of complicated skin and soft tissue infection (cSSTI) at a dose of 4 mg/kg od and for *Staphylococcus aureus* right-sided infective endocarditis (RIE) and *S. aureus* bacteraemia when associated with cSSTI or RIE at a dose of 6 mg/kg od.

Antimicrobial resistance: rising to the challenge exploring new territories to fight microbial resistance (Symposium supported by bioMérieux)

S257 Emerging resistance mechanisms: current status and future risks

D. Livermore (London, UK)*

Recent years have witnessed dramatic shifts in the prevalence and nature of antibiotic resistance. Key changes include: (i) the rise and, in some countries, subsequent decline of MRSA, also (ii) the rise of quinolone and cephalosporin resistance in Enterobacteriaceae, particularly *E. coli* and *Klebsiella* spp., with CTX-M ESBLs increasingly the dominant source of the cephalosporin resistance, (iii) the proliferation of *A. baumannii* with OXA carbapenemases and (iv) the 'loss' of fluoroquinolones against gonorrhoea, with resistance rates as high as 90% in China. Treatment shifts predicated on these problems are now exerting their own selection pressure, favouring the spread of yet further resistances. The greatest concern for hospital medicine is the emergence of Enterobacteriaceae with acquired carbapenemases, including the IMP, VIM, and NDM metallo-types, the class A enzyme KPC and the class D enzyme, OXA-48. At present, Enterobacteriaceae with these enzymes are proliferating in different parts of the world: NDM in India, OXA-48 in Turkey, VIM in Greece and KPC in the US – but the stability of this distribution is unpredictable, in view of migration and medical tourism. In general, the IMP, VIM and NDM enzymes are spread by plasmids, whilst accumulation of *K. pneumoniae* isolates with KPC enzyme largely involves dissemination of the ST258 clone. 'Loss' of the carbapenems against Enterobacteriaceae would be a public health catastrophe, in view of the lack of alternative therapies for infections due to multiresistant Gram-negative bacteria. A shift that should be of greater concern than it is presently receiving is the erosion of the anti-gonococcal activity of cephalosporins. Owing to changes to PBPs, porins and efflux it is increasingly common to see *N. gonorrhoeae* isolates with cefixime and ceftriaxone MICs of 0.12–0.5 mg/L, representing the edge of treatability, again with a lack of convenient alterative therapies. Future risks are harder to predict but potentially include transferable resistance to new anti-Gram-positive agents, and it is notable here that many soil streptomycetes can degrade daptomycin. Such organisms were the sources of several other resistance genes that have spread to mobile DNA and into clinical pathogens. But, as the saying goes, 'Prediction is very hard, especially when it concerns the future' and only two things are certain: that neither evolution nor resistance surprises have stopped yet.

S259 Individual determinants of antibiotic prescription

J.C. Lucet (Paris, FR)*

The use of antibiotics in the hospital setting is shaped by cultural and behavioural aspects as well as by clinical situation and microbiological considerations. An understanding of the determinants of antibiotic prescription is critical to explain current patterns and to devise programs to reduce inappropriate use. Physician behaviour is explained by such factors as lack of information, marketing campaigns to increase use of newer products and fear of adverse outcome with ineffective prescription. Studies of antibiotic use patterns consistently demonstrate frequent inappropriate prescribing and low adherence to antibiotic prescription (ABP) guidelines, a factor likely to increase the emergence of resistant organisms.
Behavioural aspects of the antibiotic prescription remain largely unknown. We explored beliefs and perceptions associated with measured knowledge about ABP in two French university hospitals.
Physicians in charge of ABP in inpatient hospital wards were asked to participate. Volunteers completed in a same meeting (less than 1

hour): (i) 4 case vignettes presenting infectious situations and exploring 4 aspects: whether hospitalization was needed, initiation and choice of AB, re-evaluation at day 2–3, and treatment duration and (ii) a self-reported questionnaire to collect data about their own belief and perception regarding ABP. A global score (/20) was given, combining answers to all 4 vignettes. Logistic regression identified variables independently associated with answers to case vignettes above the median global score. Of 393 eligible physicians, 206 (52%) agreed to participate: 114 senior and 92 junior physicians, comprising 100 medical physicians, 49 surgeons, 37 anesthesiologists/intensivists (AI) and 20 ER physicians. The median global score was 11.4 (IQR, 8.9–14.3). In multivariate analysis, being a surgeon (aOR, 0.14; 95% CI, 0.06–0.38, $P < 0.0001$), AI (3.33, 1.28–9.08, 0.01), perceiving inappropriate ABP as risky for the patient (3.17, 1.14–8.77, 0.03) and self-perception of ability to comply with ABP guidelines (2.74, 1.35–5.57, 0.005) were significantly associated with measured knowledge above the median score.
The participation rate was reasonably high, suggesting awareness to ABP in these hospitals. Case vignettes are a simple method to measure knowledge regarding ABP, and identified surgeons as a job profile for enhanced education. At the individual level, strengthening the perception of the risk of inappropriate ABP and self-ability to comply with guidelines may contribute to better practice with ABP. Further studies are needed to better delineate factors that can help to shape physician behaviour in antibiotic prescribing.

S260 Contribution of procalcitonin for guidance of antibiotic therapy in respiratory tract infections

P. Schuetz (Basel, CH)*

Lower respiratory tract infections (LRTI) account for almost 10% of the burden of morbidity and mortality in western countries1. LRTIs comprise a continuum of different severities of infections ranging from typically self limiting acute bronchitis, to more severe acute exacerbation of chronic pulmonary disease (AECOPD) and to life-threatening bacterial community-acquired pneumonia (CAP). Clinical signs and symptoms, and traditional laboratory parameters are unreliable to distinguish viral from bacterial LRTI. As a consequence, up to 75% of LRTI are treated with antibiotics, despite their mainly viral origin leading ultimately to emergence of bacterial resistance. To limit antibiotic overuse, rapid and more accurate differentiation of clinically relevant bacterial LRTI from other causes is key.
A novel approach to estimate the probability of a bacterial origin of LRTIs is meas-urement of serum procalcitonin (PCT) levels. PCT increases rapidly upon infection (3–6 hours) and decreases upon recovery. PCT correlates with severity and has prognostic implications. Its kinetics makes it a good marker for assessing the effectiveness of treatment, which is a prerequisite for safe guidance of antibiotic therapy. PCT levels can be used to guide antibiotic therapy in individual patients as a surrogate biomarker. Antibiotic stewardship based on PCT cut off ranges has successfully been implemented in patients with LRTI in different clinical settings. Thereby, initiation or continuation of antibiotics was more or less discouraged (<0.1 ug/L or <0.25 ug/L) or encouraged (>0.5 ug/L or >0.25 ug/L) (Figure 1). If PCT values are increased and antibiotics therapy is initiated, repeated PCT measurements are recommended and antibiotics can be discontinued using the same cut-off ranges. This clinical algorithm was prospectively tested in different intervention trials in the emergency room, in the intensive care unit, in primary care and recently, this concept has been externally validated in a large Swiss nation-wide multi centre trial including over 1350 patients. Overall, PCT-guided antibiotic stewardship reduced antibiotic prescription rate by 40–50% in patients with acute bronchitis or AECOPD; in CAP, it reduced the initial prescription rate by about 10%, but importantly shortened the duration of antibiotic therapy by 65% with a similar outcome in patients with all severities. Similarly, PCT guidance safely reduced antibiotic exposure by more than 75% in primary care patients with upper and lower RTI9. Thereby, PCT guidance significantly reduced antibiotic-related side effects, especially diarrhoea and nausea.
The septic syndrome is far too heterogeneous and complex to be reduced to a single cut-off of any surrogate marker. Biomarker must always be evaluated in the context of a careful clinical and microbiological assessment. As the kinetics of biomarkers are of particular diagnostic and prognostic interest, repeated measurements should always be performed, especially if antibiotics are withheld.
Emerging bacterial resistance to antimicrobial agents calls for more efficient efforts to reduce the unnecessary use of antimicrobial agents in self-limited and non-bacterial diseases. Embedded in a clinical algorithm, PCT-guided antibiotic stewardship offers great potential to safely and markedly reduce antibiotic exposure and antibiotic-associated side effects.

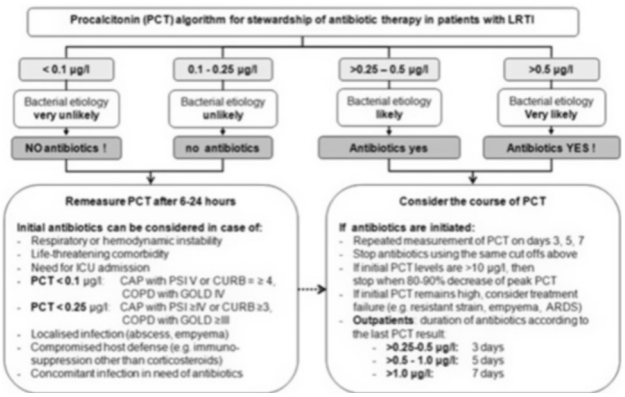

Figure 1. Clinical algorithm for antibiotic guidance in LRTI.

ESBLs and AmpCs – understand the mechanisms

S271 Current trends in epidemiology of ESBLs and acquired AmpCs

J. Pitout (Calgary, CA)*

Since 2000, *Escherichia coli* producing CTX-M enzymes (especially CTX-M-15), have emerged worldwide as important causes of community-associated urinary tract (UTIs) and blood stream infections due to extended-spectrum β-lactamase (ESBL) producing bacteria. Molecular epidemiology studies suggested that the sudden worldwide increase of multi-resistant CTX-M-producing *E. coli* (especially CTX-M-15) is mostly due to a single clone named ST131 and that foreign travel to high-risk areas such as the Indian subcontinent might play in part a role in spread of this clone across different continents. Clone ST131 that produce CTX-M ESBLs (i.e. most often CTX-M-15 but also CTX-M-3 and CTX-M-14) is associated with IncFII and IncI1 multi-resistance plasmids and certain virulence factors such as malX, ompT and usp. Community-associated acquisition and infections due to enterobacteria with plasmid-mediated AmpC β-lactamases are a relatively recent phenomenon and have been described in the UK, Canada and USA. The spread of clones in the community setting have not been described for enterobacteria that produce plasmid-mediated AmpC β-lactamases. Empiric antibiotic coverage for these resistant organisms should be considered in community patients presenting with sepsis involving the urinary tract especially in patients with certain risk factors such recent antibiotic use, residence in a long-term care facility, recent hospitalization, males older than 65 years and recent travel to a high-risk area. If this emerging public health threat is ignored, it is possible that the medical community may be forced in the near future to use the carbapenems as the first choice for the empirical treatment of serious infections associated with urinary tract infections originating from the community.

S273 The role of plasmids and bacterial clones in the spread of ESBLs and acquired AmpCs

A. Carattoli (Rome, IT)*

A multidisciplinary approach is currently applied to study the acquisition and spread of antimicrobial resistance in clinically-relevant bacterial pathogens: the established surveillance is implemented by molecular characterization of the strains by genotyping, identification of the resistance gene types, virulotyping analysis and replicon typing of the circulating plasmids. Multilocus sequence typing is also used to document the international occurrence of successful, healthcare-associated and community-acquired clones.
Many of these clones are often resistant to multiple antibiotics and multidrug resistance is often encoded by extrachromosomal elements, called plasmids. Plasmids evolve as an integral part of the bacterial genome, providing resistance genes that can be easily exchanged among bacteria of different origin and source, through the natural process of conjugation.
Particular plasmid families are playing a major role in the diffusion of ESBLs such as CTX-M, SHV, TEM and acquired AmpCs. For instance, IncFII, IncA/C, IncL/M, IncN and IncI1 plasmids carrying ESBLs are considered as "epidemic resistance plasmids", being detected in resistant bacteria of different origin and sources. The emergence of the CTX-M-15 enzyme has been associated to the spread of the highly virulent *E. coli* O25:H4-ST131 clone carrying plasmids belonging to the IncF group. Other CTX-M variants have been amplified locally, such as CTX-M-9 and CTX-M-14 in Portugal and Spain, CTX-M-3 in eastern countries associated to specific plasmid families. TEM-52 and CTX-M-1 disseminated prevalently on IncI1 plasmids among *E. coli* of human and animal origin. The identification of KPC-2-positive IncN and ColE-like plasmids in *K. pneumoniae* from USA and Colombia suggests that these plasmids could contribute to the rapid spread of the gene. Acquired AmpCs widely disseminated on IncA/C and IncI1 plasmids.
The occurrence of the particular epidemic plasmids seems tightly linked to positive selection exerted by the antimicrobial use, incrementing their prevalence compared to that observed in bacterial populations that are not pre-selected for antimicrobial resistance.
Many questions remain unanswered about mechanisms driving the successful dissemination of a specific plasmid type or a bacterial clone. However, the recognition of successful clones and plasmids is an essential first step that may lead eventually to the design of intervention strategies at preventing their spread.

S274 Mutational modifications of ESBL and AmpC activities

T. Palzkill (Houston, US)*

The class A TEM-1 and SHV-1 β-lactamases are common plasmid-encoded β-lactamases in Gram-negative bacteria. These enzymes efficiently hydrolyze pencillins and many cephalosporins but are not effective catalysts for extended-spectrum cephalosporins and thus do not provide resistance against these drugs. However, variants of TEM-1 and SHV-1 that can hydrolyze extended spectrum cephalosporins have evolved in clinical isolates over the last two decades, which has limited treatment options. These evolved enzymes, termed extended-spectrum β-lactamases (ESBLs), contain from one to five amino acid substitutions that result in increased catalytic efficiency of the enzymes for hydrolysis of extended spectrum cephalosporins. Site-directed and random mutagenesis studies have revealed that the amino acid substitutions commonly found in the evolved variants increase the catalytic efficiency for hydrolysis of extended spectrum cephalosporins and, in some cases, stabilize the evolved enzymes. The evolution of high level resistance involves a process of mutational changes in the active site that increase catalysis but often decrease protein stability. This, in turn, leads to the acquisition of secondary mutations that increase the *in vivo* half life of the enzyme. The interactions between amino acid substitutions and the resultant impact on the evolution of ESBL activities will be discussed.
AmpC β-lactamases are highly efficient for hydrolysis of many cephalosporins, however, they exhibit lower activity towards extended-spectrum cephalosporins. Nevertheless, AmpC mediated resistance to extended-spectrum cephalosporins can occur due to high levels of enzyme production or through mutations in the enzyme that result in increased hydrolysis of the drugs. Several amino acid substitutions as well as small insertions and deletions in AmpC enzymes have been reported to increase resistance to extended spectrum cephalosporins and have been associated with increased hydrolysis of these antibiotics. The mutational changes occur in regions in the vicinity of the active site and may act by increasing access for the bulky oxyiminocephalosporins. In addition, random mutagenesis experiments have revealed positions where amino acid substitutions alter the substrate specificity of the enzyme. The impact of amino acid substitutions on the evolution of extended spectrum cephalosporin hydrolysis by AmpC enzymes will be discussed.

Non-tuberculous mycobacterial infections

S275 Non-tuberculous mycobacterial pulmonary infections

E. Tortoli (Florence, IT)*

The lung can be easily affected by inhalation of aerosolized mycobacteria and is by far the most frequent site of human mycobacteriosis.
In HIV-negative patients, the disease is undistinguishable from tuberculosis and is characterized by very slow progression. The manifestations range from absence of symptoms to cavitary disease, and X-ray may reveal fibrosis, upper lobe cavitation, nodular or parenchymal opacity, and pleural thickening. The most affected are elderly patients with predisposing pulmonary conditions (e.g. silicosis, obstructive pulmonary disease, pneumoconiosis, previous tuberculosis, bronchiectasis or cancer). The symptoms include cough, fever, weight loss, weakness and respiratory insufficiency. The NTM most frequently responsible for disease belong to the *Mycobacterium avium* complex; in Europe, infections caused by *Mycobacterium xenopi* and, particularly in northern countries, those caused by *Mycobacterium* malmoense are also quite frequent whereas, in the USA, infections caused by *Mycobacterium kansasii* are more prevalent.
In AIDS patients the radiographic picture is often normal or may reveal mediastinal or hilar adenopathy, and the progression of disease is very rapid. The most frequent symptoms are cough, fever and weight loss. The patients have usually a CD4 lymphocyte count lower than 100/μL. In recent years, the widespread use of highly active anti-retroviral treatments has dramatically reduced the frequency of mycobacterial diseases, including the pulmonary ones, in HIV-positive patients.
Recently pulmonary infections due to NTM have increasingly been reported in patients with cystic fibrosis.
Because of the high risk of contamination of the sputum by environmental mycobacteria, it is estimated that less than 30% of the isolations of NTM from the respiratory tract have clinical relevance. The strict observance of the criteria of the American Thoracic Society is essential for a correct diagnosis.

S276 Buruli ulcer disease: epidemiology, clinical manifestations, diagnosis, treatment

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Buruli ulcer (BU), caused by *Mycobacterium* ulcerans is the third most common human mycobacteriosis worldwide after tuberculosis and leprosy.
BU is endemic in rural wetlands of tropical countries of Africa, America, Asia and Australia but remains uncommon in non-African countries.
A few cases have been reported in non-tropical areas. Incidence rates vary greatly by continent, country, and in areas within a country. Known incidence rates currently are highest in West Africa.
Children 15 years old or younger account for approximately 75% of cases. Risk factors include tropical climate, exposure to stagnant water,

unprotected water sources, hygiene, trauma to skin and HIV infection. *M. ulcerans* disease presents a spectrum of forms related partly to patient delay in admission to hospital. Estimated incubation periods range from 2 weeks to several years.

The nonulcerative forms represent the first stages of the disease. Early stages are often ignored by the patients and may sometimes heal spontaneously.

The clinical diagnosis can be difficult even for experienced health professionals, hence the importance of microbiologic confirmation.

Four laboratory tests are currently in use:

1. Direct smear of exudates from ulcerated lesions in situ, or fine needle aspirates (FNA) from nonulcerated lesions, or biopsy specimens
2. *In vitro* cultivation of *M. ulcerans* from exudates, FNA or biopsies.
3. Gel-based or quantitative real-time PCR targeting IS2404.
4. Histopathologic examination (important in both BU diagnosis and differential diagnosis).

Numerous other infectious and noninfectious diseases and neoplasms are diagnosed among clinically suspected cases of BU. Tropical phagedenic ulcer is the most common diagnostic problem.

Options for therapy for BU are surgery, antibiotics, or combinations of both. Antibiotic therapy for all forms is gaining acceptance. The World Health Organization (WHO) recommends, if not contraindicated, the directly observed use of at least an 8-week course of oral rifampin and streptomycin by intramuscular injection. This antibiotic treatment may also be combined with surgery. Other antibiotic regimens, especially those administered completely orally, are under evaluation.

Severe ZN positive lesions (large ulcers and disseminated forms) should receive specific antibiotherapy and should be surgically treated without delay to avoid deterioration of lesions and possible bone dissemination.

S277 Non-tuberculous mycobacterial infections related to cosmetic and medical procedures and acupuncture

A. Carbonne, F. Brossier, I. Arnaud, I. Bougmiza, E. Cambau, J.P. Meningaud, V. Jarlier, E. Caumes, P. Astagneau (Paris, FR)*

Objectives: Mesotherapy is an increasing used technique involving subcutaneous injections of minute quantity of various medical drugs for cosmetic or rheumatism purposes. This practice was already reported to be related to infection risk.

In January 2007, a general practitioner notified to the health authorities and the regional centre for infection control a cluster of subcutaneous infections due to non-tuberculosis Mycobacteria (NTM) following mesotherapy. An epidemiological investigation was performed to describe the outbreak, to identify the source and the mechanism of contamination and to determine risk factors.

Methods: The case definition was based on typical clinical subcutaneous lesion associated with positive specimens for NTM. An assessment practice study was performed to determine potential risk factors to be tested in a comparative epidemiological study. Data were collected including schedules of outpatient visits, localisation of injections, and type of injected products. Tap water of the medical examination room was sampled for search of Mycobacteria. *Mycobacterium chelonae* strains were compared using Pulsed Field Gel Electrophoresis (PFGE).

Results: Overall, 16 cases were identified among 105 outpatients (attack rate: 15.2%), including 11 positive for *Mycobacterium chelonae* and 2 for *M. fredericbergensen. M. chelonae* was found in the tap water. Assessment practice study identified inappropriate cleaning and rinsing of the multiple injection device (automatic repetitive machine) using tap water which was likely to be the source of NTM contamination. Indeed, PFGE *M. chelonae* strains patterns were similar between patients and tap water samples. In the univariate analysis, NTM infection incidence rate was higher in patients having Monday or Thursday visits, being at the 2nd rank during the session, having cosmetic purpose for weight loss or more injections on abdomen, upper leg or hip. In the multivariate analysis, being at the 2nd rank during the session(Odds ratio = 3.1 [1.0–9.6]) and having a higher rate of visits on Monday or Thursday (Odds ratio = 9.7 [1.2–77.8]) remained the only independent risk factors of NTM infection.

Conclusion: This investigation highlights that failure in disinfection of injecting material could generate severe infections with highly resistant bacteria related to non regular medical cares. Efforts should focus on control of hygiene practices in non hospital settings based on appropriate guideline recommendations.

S278 Management of non-tuberculous mycobacterial lymphadenitis in children

J. Amir (Petah Tiqwa, IL)*

The spectrum of clinical manifestations caused by nontuberculous mycobacteria (NTM) in immunocompetent individuals comprises three major categories: lymphadenitis, pulmonary infections and skin/soft tissue infections. Lymphadenitis due to NTM strikes mainly young children whereas pulmonary and skin/soft tissue infections are common in adults. The frequency of NTM lymphadenitis has increased over the past few decades. Diagnosis is based on clinical presentation, PPD skin test and bacterial isolation from nodal aspiration or incision. *Mycobacterium scrofulaceum* was the most common cause in the 1970s, replaced by *M. avium*-intracellulare complex (MAC) and *M. haemophilum* in last two decades. Management options are surgery, antibiotics or "observation only". Complete excision of the infected lymph node has been considered the optimal therapy by most researchers, however, it is associated with various side effects such as unacceptable scarring with or without keloid formation, wound breakdown, secondary staphylococcal infection and facial nerve paresis. Most facial nerve damage is transient, although in approximately 2% permanent palsy developed. Incision and drainage is performed when the lesions are too large to be excised. Few retrospective case series have demonstrated superiority of complete excision over incision and drainage. Pharmacologic therapy with clarithromycin alone, or combined with other antimycobacterial agents such as rifampicin, rifabutin, or ethanbutol have been reported. On the other hand, there are no controlled clinical trials showing the efficacy of chemotherapy versus placebo. Very few cases of "observation only" in children with NTM lymphadenitis were reported in the past. A recently published study described the natural history of 92 immunocompetent children with cervical NTM lymphadenitis. In most cases, the skin over affected lymph nodes underwent violaceous changes, with discharge of purulent material for 3–8 weeks. Total resolution was achieved within 6 months in 71% of the patients, and within 9–12 months in the remainder. No complications were observed, and at 2 years follow-up, a skin-colored flat scar in the region of the drainage was noted.

In conclusion, The optimal therapy for this condition is still controversial. Nevertheless, it seems that antibiotics are not very effective in treating immunocompetent children. A randomized, controlled trial examining surgical excision versus spontaneous healing is warranted.

Treatment and prophylaxis of malaria

S279 Why malaria prophylaxis sometimes fails?

V. Krcmery, J. Sokolova, L. Seng Duong (Bratislava, Trnava, SK)*

Reasons for antimalarial prophylaxis failure include:

i. poor adherence to antimalarial agents
ii. malabsorption of antimalarial drugs
iii. interactions with other medications
iv. adverse reaction leading to treatment interruptions
v. underdosing the patient
vi. *in vitro* resistance

In case of clinical failure standby treatment is recommended, if no health infrastructure is available. Commonly used antimalarials for prophylaxis in travelers include mefloquine or atovaquon-proguanil. In prophylaxis failure therapy with artemisin based combination drugs (ACT) is indicated. Special conditions exist for IPT (Intermittent Preventive Treatment) in pregnant women in endemic areas and children. For long term travelers/residents prophylaxis in hyperendemic areas is usually ineffective.

S282 Artemisinin resistance

H. Noedl (Vienna, AT)*

Within the past 10 years virtually all malaria-endemic countries have officially adopted artemisinin-based combination therapies (ACTs) as first or second line therapy for the treatment of *P. falciparum* malaria. Artemisinins have become the most essential class of antimalarials, their impact comparable only to that of chloroquine in the mid 20th century. Spreading artemisinin resistance could therefore have a devastating impact on malaria control efforts throughout the malaria-endemic world. In the current situation losing a single class of antimalarial drugs to resistance may severely impact the ability of many countries to treat falciparum malaria.

The concept of artemisinin resistance has been a contentious one for many years, with some authorities suggesting that it was unlikely to arise in the first place. However, recent data indicate that the first cases of genuine artemisinin resistance have already emerged in western Cambodia. We may already be losing artemisinins in selected parts of the world.

Our data showing individual parasite isolates resistant to high doses of artemisinins, prolonged parasite clearance times, and reduced *in vitro* drug response indicate that this phenomenon is as yet limited to a relatively small proportion of the parasite isolates and probably also to a relatively small area in Southeast Asia.

Once it starts spreading, resistance to artemisinin derivatives, currently the most essential antimalarial drugs, could very well be the most devastating event in the history of malaria control in the 21st century. Artemisinin-resistant malaria is a new emerging disease that will require new treatment and control strategies to limit the impact and spread of resistance to the rest of the malaria-endemic world.

Microbial colonization of respiratory tract: does it always predict a nasty outcome?

S285 Clinical significance of *Pneumocystis* colonization

A. Morris (Pittsburgh, US)*

Pneumocystis pneumonia has long been recognized as a cause of morbidity and mortality in immunocompromised populations, particularly those with human immunodeficiency virus (HIV) infection. *Pneumocystis* colonization is defined as detection of the organism or its DNA without signs or symptoms of pneumonia. Sensitive molecular techniques such as polymerase chain reaction are frequently utilized to identify colonization. Accumulating evidence suggests that colonization with *Pneumocystis* may be an important clinical phenomenon. The clinical significance of colonization is not yet fully understood, but it may be important for several reasons. Colonized persons may be at risk of developing *Pneumocystis* pneumonia. Even if colonized individuals do not themselves develop *Pneumocystis* pneumonia, they might transmit the organism to others. Exposure to *Pneumocystis*-colonized animals leads to colonization of normal animals and to the development of clinical disease in immunosuppressed animals. Colonization in persons receiving long-term anti-*Pneumocystis* prophylaxis may also lead to the development of mutations that have been associated with drug resistance. Furthermore, *Pneumocystis* may play a role in progression of lung diseases such as chronic obstructive pulmonary disease (COPD). The presence of *Pneumocystis* in the lungs, even at low levels as seen in colonization, produces inflammatory changes similar to those seen in COPD, with increases in the numbers of neutrophils and cytotoxic CD8+ lymphocytes. Colonization with *Pneumocystis* has been demonstrated in HIV-infected subjects, and HIV-infected smokers are particularly susceptible to *Pneumocystis* colonization regardless of CD4 cell count or use of *Pneumocystis* prophylaxis. *Pneumocystis* colonization is also increased in non-HIV-infected patients with COPD and is directly related to the severity of the disease. Models of *Pneumocystis* colonization in mice with smoke exposure and in non-human primates infected with an HIV/simian immunodeficiency virus chimera demonstrate the development of COPD-like changes. These studies suggest that treatment or prevention of *Pneumocystis* colonization in at-risk populations may be a novel therapy for COPD.

Virus oncogenesis: mechanisms and clinical aspects

S287 EBV-associated tumours

D.A. Thorley-Lawson (Boston, US)*

Epstein–Barr virus is a human herpesvirus that is known to infect and efficiently drive the activation and proliferation of resting B cells *in vitro*. It achieves this through the expression of nine latent proteins. However, *in vivo* EBV persists in a benign, dormant state in resting memory B cells. The link between these two observations is that *in vivo* EBV uses its latent proteins to activate newly infected cells in lymphoid tissue so that the cells can then differentiate through the germinal center to become resting memory cells. In doing so the virus infected cells mimic the normal process whereby antigen activated B cells become long term memory cells. At each stage of the process EBV uses different combinations of latent proteins. If at any stage the infected cell is unable to progress into the resting memory state it is at risk of becoming a tumor expressing the pattern of latent proteins characteristic of its particular stage of infection. Thus EBV is associated with several human malignancies including Burkitt's lymphoma, Hodgkin's disease, immunoblastic lymphoma, nasopharyngeal carcinoma and gastric carcinoma. Each of these tumors expresses a different pattern of latent proteins reflecting their origins from specific stages of the viral life cycle. Recently it has been shown that EBV also expresses ~40miRNAs in latently infected cells. miRNAs are thought to play critical regulatory functions in normal and malignant cells but the roles of the EBV miRNAs remain unclear. It is also unknown if they are expressed in a tumor specific pattern like the latent proteins. In this presentation I will:

1. Discuss the mechanism of how EBV establishes persistent infection in memory B cells including recent evidence on how EBV usurps the germinal center process to gain access to the memory B cell compartment.
2. Detail how the model of EBV persistence explains the origin of the EBV associated tumors especially the lymphomas.
3. Demonstrate tumor specific expression profiles of miRNAs and a possible functional significance of these expression patterns.

S290 Polyoma virus-associated tumours

J.M. Pipas (Pittsburgh, US)*

Some polyomaviruses induce tumors in their natural hosts or in test animals. Tumorigenesis by these viruses is effected by a number of different mechanisms depending on the specific virus. Studies with simian virus 40 have shown that this virus targets key cellular regulatory proteins, in particular the tumor suppressors pRb and p53 to stimulate cell proliferation and to block cell death. Our laboratory uses a combination of genetics, proteomics, gene expression arrays and transgenic and knockout mice to understand how the action of SV40 proteins on these cellular targets leads to tumorigenesis. This talk will review this research and present a model for SV40-induced transformation and tumorigenesis.

Innate immunity to pathogens: from genes to cells

O291 The role of staphylococcal Panton-Valentine leukocidin during lung inflammation *in vivo*

A. Zivkovic, O. Sharif, K. Stich, M. Biaggio, B. Doninger, I. Mesteri, S. Knapp (Vienna, AT)*

Objective: Panton-Valentine Leukocidin (PVL) positive *Staphylococcus aureus* is an emerging pathogen associated with highly lethal necrotizing pneumonia. PVL is a bi-component β-barrel pore-forming toxin that has been shown to cause cell death in neutrophils. However, the precise mechanisms leading to necrotizing pneumonia are not fully understood and the role of PVL herein is controversial. We therefore aimed to investigate the mechanism by which PVL might contribute to the development of severe lung inflammation.
Methods: Recombinant PVL was generated and its cytotoxic properties were verified by patch clamp and FACS. The inflammatory potential of PVL was tested on alveolar macrophages using mciroarray profiling, PCR and ELISA. Signaling pathways were investiagted using western blots and electromobility shift assays. The *in vivo* role of PVl was then studied in a murine pneumonitis model, in which lung tissue and lavage fluid was assessed for cell-influx and cytokine/chemokine release.
Results: PVL rapidly induces pore formation and death of neutrophils. In contrast, alveolar macrophages were found less sensitive to the toxic effects and only succumbed after prolonged incubation. Microarray assays performed 1 h after addition of PVL to alveolar macrophages revealed the selective induction of 29 genes upon stimulation. Bioinformatic analysis disclosed the upregulation of NFkB target genes. These data were verified by PCR and on protein levels and suggest that PVL selectively induces NFkB associated inflammation *in vitro*. Inhibitor studies and gene reporter assays further confirmed this finding. Furthermore, PVL-induced lung inflammation in mice confirmed the inflammatory potential of PVL, as illustrated by a rapid influx of neutrophils and enhanced levels of pro-inflammatory cytokines and chemokines within the pulmonary compartment.
Conclusions: Our data demonstrate that PVL, beside its pore-forming properties on neutrophils, is able to strongly induce inflammation via NFkB activation in alveolar macrophages, leading to pneumonitis *in vivo*.

O292 Serum IgG antibodies strongly augment lipoteichoic acid induced immune activation in human peripheral blood

S. Sigel, S. Bunk, D. Metzdorf, T. Hartung, S. Knapp, S. von Aulock (Vienna, AT; Constance, DE)*

Objective: *Staphylococcus aureus* (*S. aureus*) is an important human pathogen that causes diverse diseases ranging from innocuous skin infections to often fatal forms of sepsis. During the initial phase of infection specific molecular patterns of *S. aureus* are recognized by innate immune receptors of the host leading to the induction of inflammatory responses. Among these patterns lipoteichoic acid (LTA), a component of the Gram-positive cell wall, represents a potent immunostimulatory molecule that is able to elicit cytokine release from human peripheral blood in a TLR2/6 dependent manner. Since in contrast to our data, LTA of a mutant *S. aureus* strain lacking lipoproteins (delta lgt-LTA) has been described by other investigators to be immunobiologically inactive, we investigated the functional requirements for the delta lgt-LTA mediated activation of innate immune responses.
Methods: Using an *ex vivo* model based on human peripheral blood cells the conditions and cofactors required for the recognition of delta lgt-LTA by innate immune cells were investigated.
Results: In this study we demonstrate that delta lgt-LTA induced immune activation critically depends on the immobilization of LTA and the phagocytic activity of blood cells as well as the presence of human serum components, which was also observed for LTA of the respective wild type *S. aureus* (wt-LTA) albeit with a lower degree of significance. Under experimental conditions, conducive to optimal LTA stimulation, we found no differences between the immunostimulatory capacity of delta lgt LTA and wt-LTA arguing for a limited contribution of possible lipoprotein contaminants to wt-LTA mediated immune activation. Interestingly, we found that LTA specific IgG antibodies, here detected in human sera at varying levels, can bind to immobilized delta lgt-LTA and thereby strongly augment delta lgt-LTA mediated immune activation in human peripheral blood.
Conclusion: Our results not only suggest a novel mechanism for delta lgt-LTA mediated immune activation in human blood cells that involves an opsonization-dependent uptake and recognition process of LTA but also provide a conclusive explanation for the controversial findings obtained in previous experiments comparing the immunostimulatory capacity of delta lgt-LTA and wt-LTA.

O293 Hypoxia amplifies inflammatory signalling in response to *Pseudomonas aeruginosa* in pulmonary epithelial cells

B. Schaible, C. Taylor, K. Schaffer (Dublin, IE)*

Objectives: Cystic Fibrosis (CF) is an autosomal recessive disorder with an incidence of 1 in 2500 in Caucasians. *Pseudomonas aeruginosa (P. aeruginosa)* is the predominant bacterial pathogen in CF and grows within a hypoxic biofilm in the CF lung causing chronic infection and associated inflammation. In this study we have investigated the effects of hypoxia on the activity of the proinflammatory transcription factor NF-kappaB in alveolar epithelial cells in response to stimulation with *P. aeruginosa*.
Methods: A549 lung epithelial cells were incubated with heat inactivated *P. aeruginosa* or specific TLR ligands (TLR2: Pam3CysSK4, TLR4: LPS, TLR5: flagellin) at 21% and 1% atmospheric oxygen. Activation of NF-kappaB was determined by immunoblotting of nuclear of p65 (NF-kappaB family member) and cytosolic Cox2 (target gene of NF-kappaB) as well as by luciferase activity, wereby A549 cells were transfected with a NRE-luciferase plasmid beforehand.
Results: Incubation of cells with heat inactivated *P. aeruginosa* clearly increased nuclear p65 protein level and luciferase activity in a manner that was profoundly amplified by either epithelial cell hypoxia or hypoxic bacteria. Stimulation of innate immunity in alveolar epithelial cells is largely mediated by three toll-like receptors (TLR2, TLR4 and TLR5). Incubation of cells with toll-like receptor specific agonists, primarily flagellin for TLR5, increased NF-kappaB level in a manner which was also amplified by hypoxia.
Conclusions: These data indicate that microenvironmental hypoxia is a major determinant of the pulmonary epithelial inflammatory response during *P. aeruginosa* infection. Further investigations of signaling molecules participating in this response will reveal new targets for anti-inflammatory therapy in Cystic Fibrosis.
This work is supported by grants from IRCSET Embark Initiative and Science Foundation Ireland.

O294 Nalp3 and ASC are not required for caspase-1 mediated antifungal host defence in disseminated candidiasis

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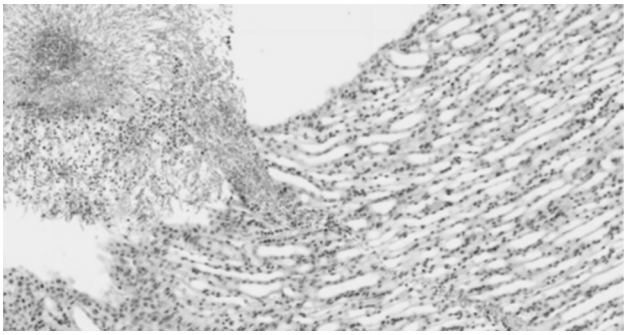
Objectives: Contradictory data have been reported regarding the role of the inflammasome in anti-*Candida* host defense. In order to address these discrepancies, we investigated host defense against disseminated candidiasis in knock-out mice defective in the various components of the inflammasome.
Methods: Mice deficient in caspase-1, ASC, Nalp3 or P2X7 were infected intravenously with *C. albicans*. Survival, fungal outgrowth in the organs, histology, and modulation of cytokine production were compared in these mouse strains with the wild-type C57/Bl6 control mice. PBMCs from healthy volunteers with or without reactive oxygen species (ROS) inhibitor and PBMCs from patients with chronic granulomatous disease (CGD) that are deficient in ROS production were stimulated with *C. albicans*.
Results: Caspase-1–/– mice and ASC–/– mice had a decreased survival during disseminated candidiasis (50%) compared to the control mice

(87%). Caspase-1–/– mice had a 100-fold increase in fungal loads in the kidneys of the deficient animals and showed preferential growth of hyphae in the pyelum of the caspase-1–/– mice accompanied by remarkbly little inflammation (Figure). In contrast, ASC–/– mice did not have higher fungal loads, but they showed a significantly stronger inflammatory reaction in the kidneys. ASC–/–splenocytes showed a higher TNF production after *Candida* stimulation. NLRP3–/– and P2X7–/– did not display an increased susceptibility to disseminated candidiasis. Local bioactive IL-1β and INFγ *in vivo* was only affected in the caspase-1–/– mice, but not in the ASC–/–, NLRP3–/– or P2X7–/– animals. In addition, caspase-1 was constitutively active in ROS deficient monocytes and *C. albicans* induced IL-1β production was not reduced in the absence of ROS.

Conclusions: Caspase-1-dependent processes are important in anti-fungal host defense during *Candida* sepsis. In contrast to current thinking, these process are not dependent on the inflammasome components, ASC, NLRP3 and the ATP receptor P2X7. These data confirm previous studies in human monocytes showing that caspase-1 activation during *Candida* infection does not require pathogen-mediated in flammasome activation. ASC plays an important role in disseminated candidiasis, but unexpectedly seems to have a different function *in vivo*, specifically by regulating TNF production and local inflammation in the organs. Finally, ROS did not play a critical role in inflammasome activation and *Candida*-induced IL-1β production.



O295 Amphotericin B mediates killing in *C. neoformans* through induction of oxidative burst rather than through pore formation at the membrane

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Objectives: Amphotericin B (AmB) is an antifungal drug widely used for the treatment of fungal infections, such as cryptococcal meningitis, which is caused by the encapsulated fungal pathogen *Cryptococcus neoformans*. AmB binds to ergosterol and forms pores at the membrane, resulting in cell death. However, other reports indicate that AmB also acts as an oxidant. In this work we have studied the effect on AmB on *C. neoformans.*

Methods: We have compared three viability methods: XTT assay, prodium iodide staining, and colony forming units enumeration. XTT is reduced in the mitochondria by living cells, producing a compound that is quantified spetrophotometrically. Propidium iodide is a DNA-binding fluorescent compound that only penetrates in the cells once they lose the membrane integrity. Finally, CFUs enumeration estimates the ability of the cells to replicate and form viable colonies.

Results: While AmB inhibited the formation of colonies at concentrations above 0.5–1 mg/L, the cells did not become permeable to propidium iodide in the same conditions, suggestin that AmB has other effects on *C. neoformans* different than pore formation. When viability was measured using the XTT assay, a correlation with the CFUs results was observed, confirming that AmB exerts its killing effect intracellularly. However, we also observed a "paradoxical effect" in which cells treated with high AmB concentrations (4–16 mg/L) produced higher levels of reduced XTT than cells treated with intermediate AmB concentrations (0.25–1 mg/L). Since this "paradoxical effect did not correlate with CFUs appearance, we argued that it might reflect an induction of the electron transfer in the mitochondria, as a consequence of an increased oxidative burst induced by AmB. So we measured the amount of reactive oxygen species (ROS) in the cells using dihydrofluorescein, a compound that when is attacked by free radicals releases fluorescence. Our results demonstrated that AmB induced a strong release of ROS in the cells, which correlates with the metabolic inactivation measured by the XTT assay.

Conclusions: Although AmB can bind to ergosterol and produce pores at the membrane, our results indicate that this antifungal drug induces killing in *C. neoformans* mainly through an induction of a strong oxidative burst. These findings confirm that Am B has multiple effects on the cells, which suggests an explanation for the low antifungal resistance to this compound observed among clinical isolates.

O296 Characterization of class I-restricted epitopes of *Trypanosoma cruzi* HSP70 protein recognized by Chagas' disease patients

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Objectives: The protozoan parasite *Trypanosoma cruzi* is the aetiological agent of Chagas disease or American trypanosomiasis, which affect near 20 million people with severe consequences in terms of morbidity and mortality. The host's ability to generate a parasite-specific immune response plays an essential role in the control of the evolution and the severity of the disease. In particular, efficient CD8+ T cell responses are required for the immune control of the infection. Heat shock proteins have been characterized as immunodominant CD8+ antigens in several bacterial infections and tumours, but not still in parasitic diseases. The aim of this work is to know whether *T. cruzi* HSP70 is processed and presented to CD8+ T lymphocytes during natural infection.

Methods: Peptides containing HLA-A*0201-binding motifs were selected using bioinformatics, and their binding affinity were evaluated using T2-binding assays. HSP70-immunized or *T. cruzi* infected C57BL-A2/Kb transgenic mice were used to predict immunodominant epitopes within the protein by cytotoxicity assays. The recognition of candidate peptides by circulating lymphocytes from chagasic patients were assessed through IFN-g secretion tests.

Results: Thirty peptides were selected as putative HLA-A*0201 binders and synthesized. Of these, 16 candidates were selected and their recognition was tested by splenocytes of C57BL-A2/Kb mice immunized with HSP70 protein or, alternatively chronically infected with *T. cruzi.* No significative peptide recognition was observed in non-infected or non-immunized animals, and two peptides were significatively recognized by both immunized and infected mice. When the recognition of these two peptides by circulating lymphocytes of chagasic patients was tested by IFN-g secretion assays, both peptides were well recognized by chagasic patients in different stages of the disease.

Conclusion: The data above demonstrate that *T. cruzi* HSP70 is an immunodominant CD8+ epitope, which is naturally processed and presented during Chagas disease progression. CD8+ T cell response against microbial HSP70 has been shown to be protective in other infections like tuberculosis. Since currently no vaccine against Chagas disease is available, the characterization of new parasite antigens providing protective responses will be of great value in order to fight against *T. cruzi* infection.

O297 Differential induction of *in vitro* CD4+/CD8+ T-cell responses by live vs. killed *Leishmania major*

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Objectives: Different clinical trials of killed *Leishmania* vaccines showed a limited efficacy compared to inoculation of live *Leishmania major* (leishmanization=LZ) against cutaneous leishmaniasis (CL). The reason for the discrepancy in efficacy of live and killed vaccines is not yet

known, *in vivo* and *in vitro* studies on T-cell function against live/killed stimulatory effect might provide valuable information.
Methods: Nine leishmanin skin test (LST) positive donors with history of self healing CL (HCL) and seven healthy LST negative donors were included in this study. CD4+/CD8+/CD14+ cells were isolated from peripheral blood. 5,6-carboxyfluoescein diacetate succinimidyl ester (CFSE) labeled CD4+ or CD8+ lymphocytes were cultured in the presence of 1:10 of mitomycin treated autologous monocytes and 100 micro g/ml killed *Leishmania* lysate (KLL) or 2.5×10^5/ml live *Leishmania major* (LLM). Cells were harvested after 7 days of incubation at 37ºC and analysed for proliferation using flow cytometry. Culture supernatants were collected on day 3 for IL-5, IL-10, IL-13 and IFN-g titration using sandwich ELISA method.
Results: In HCL volunteers, upon stimulation with KLL, the number of proliferated CD4+ or CD8+ T cells (at division 4) was significantly more than unstimulated cells ($P < 0.001$) or LLM stimulated cells ($P < 0.05$) or cells from controls ($P < 0.05$ for CD4+ and $P < 0.001$ for CD8+ cells). Cells from HCL donors showed a significantly higher IL-10 production to LLM stimulation compared with KLL stimulation ($P < 0.001$ for CD4+ and $P < 0.0005$ for CD8+ cells) or comparing controls ($P < 0.05$ for CD4+ and $P < 0.001$ for CD8+ cells). Stimulation of CD4+ T cells with LLM ($P < 0.001$) or KLL ($P < 0.05$) induced a significantly higher IFN-g production compared to cells from controls, but LLM induced significantly more IFN-g than KLL ($P < 0.05$). On the CD8+ compartment, a significantly higher IFN-g production was observed when the cells were stimulated with LLM ($P < 0.05$).
Conclusion: While killed *Leishmania* induced more proliferation response in purified T cells of HCL volunteers, live *Leishmania* induced cytokine production in T cells without a significant induction of proliferation. The results from healed CL volunteers in this study might be implicated in further investigations on T-cell function against live/killed *Leishmania* vaccination *in vivo*.

O298 Genetic variants of dectin-1 and susceptibility to infections with *Candida* spp.

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Objectives: Systemic and oropharyngeal candidiasis represent a cause of high morbidity especially in immunocompromised hosts, with risk varying significantly between individuals. Recognition of *Candida albicans* is mediated through pattern recognition receptors such as the β-glucan receptor dectin-1. We recently identified an early stopcodon single nucleotide polymorphism (SNP) Y238X in dectin-1 that is associated with the loss of β-glucan recognition (N Engl J Med. 2009;361:1760–7).
Methods: We assessed whether the presence of the dectin-1 Y238X SNP was associated with an increased susceptibility to *Candida* infections in several clinical settings: i. in 365 American and Dutch patients with candidaemia compared to 351 non-infected American and Dutch controls; ii. in 142 Dutch patients undergoing haematopoietic stem cell transplantation (HSCT); and iii. in 170 Greek HIV-infected patients.
Results: Dectin-1 Y238X SNP was strongly correlated with *Candida* colonization in HSCT patients: 34% of the individuals bearing the wild-type allele were colonized with *Candida*, compared to 85% of the individuals bearing the mutant allele ($p < 0.005$), necessitating more frequent use of fluconazole treatment ($p < 0.01$). Functional assays demonstrated a loss-of-function phenotype of the SNP, as shown by the decreased cytokine production by immune cells bearing this SNP. In contrast, the dectin-1 Y238X SNP did not influence susceptibility to candidaemia or that of oropharyngeal candidiasis in HIV-infected patients.
Conclusions: The dectin-1 Y238X SNP is non-redundant for the recognition of *C. albicans*, and influences mucosal *Candida* colonization. In contrast, lack of functional dectin-1 is not accompanied by an increased susceptibility to bloodstream *Candida* infections.

O299 Single nucleotide polymorphisms in the PTX3 gene do not affect susceptibility to meningococcal disease but influence plasma concentrations during meningococcal septic shock

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Objectives: Meningococcal disease is an important cause of morbidity and mortality world-wide. Pentraxin 3 (PTX3) is an important element of the innate immune system. Experimentally, PTX3 binds meningococci and activates complement and PTX3 is elevated in meningococcal septic shock. This prompted us to evaluate the relevance of single nucleotide polymorphisms (SNPs) in the gene for PTX3 in relation to susceptibility to meningococcal disease, disease manifestation and plasma concentrations of PTX3 during septic shock.
Methods: We studied a sample of 115 case-parent triads and 153 individual patients that had been admitted to the ICU with meningococcal disease. From 21 patients PTX3 plasmaconcentrations during meningococcal disease were determined using ELISA. PTX3 SNPs rs2305619 and rs3816527 were analysed using PCR.
Results: Minor allele frequencies were 0.468 (G) for rs2305619 and 0.451 (C) for rs3816527. Allele frequencies did not differ significantly from Hardy-Weinberg equilibrium. The SNPs rs2305619 and rs3816527 are in linkage disequilibrium.
TDT analysis showed no association of the SNPs rs2305619 and rs3816527 in the gene for PTX3 with meningococcal disease. Also no preferential transmission of these SNPs was found in the subgroups of patients with meningitis, shock, shock and meningitis or bacteraemia. Patients with meningitis had a somewhat higher prevalence of the A allele in rs2305619 and the C allele in rs3816527, but this was not significant. In addition, no differences were found for number of days spent in ICU or disease severity. During active meningococcal infection, in patients with meningococcal septic shock, peak plasma concentration of pentraxin 3 appeared to be dependent on PTX3 genotype. Patients homozygous for the A allele in rs2305619 had higher peak plasma concentrations of PTX3 than other individuals ($P = 0.05$). Instead, patients homozygous for the A allele in rs3816527, had lower peak plasma concentrations than AC and CC individuals, this approached significance ($P = 0.09$) (Figure 1).
Conclusions: These findings indicate that in patients with septic shock PTX3 plasmaconcentrations are in part dependent on PTX3 genotype for the rs2305619 and rs3816527, but these SNPs do not affect susceptibility to meningococcal disease.

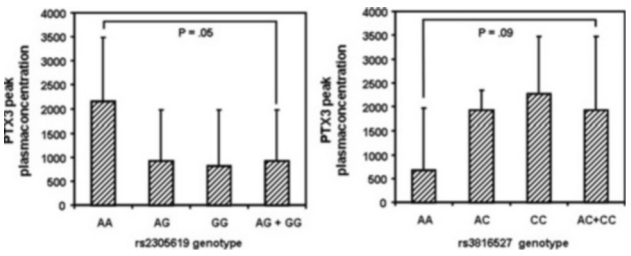

Figure 1. Pentraxin 3 peak plasma concentrations during meningococcal septic shock according to PTX3 genotype. Medians and interquartile ranges are presented. P value by Mann–Whitney test.

O300 Mannose-binding lectin and tuberculosis infection: a meta-analysis

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Objective: Resistance to tuberculosis has been proposed as a mechanism for selection for high rates of mannose-binding lectin (MBL) deficiency. Mechanistically, low levels of this pattern recognition receptor may lead to relatively lower degrees of phagocytosis of *Mycobacterium tuberculosis* and protect against TB however evidence to date has been

conflicting. We aimed to systematically review published literature on the effect of MBL2 genotype and/or MBL levels in tuberculosis infection, and perform meta-analysis considering its effect on susceptibility to infection.
Methods: We performed a systematic literature review of Medline and PubMed from 1966 to August 2009 to identify articles considering the effect of MBL2 genotype and/or MBL levels on tuberculosis infection. All human trials that included subjects with confirmed tuberculosis and either MBL serum levels or MBL2 genotypes were selected and included in meta-analysis. The frequency of MBL2 structural gene variants, referred to collectively as 'O' (wild type = 'A') was extracted from the published data in each paper. While out of keeping with contemporary knowledge, in this retrospective meta-analysis we have had to define MBL deficient genotypes as A/O or O/O. To consider evidence of publication bias, we prepared funnel plots of the studies included in the final analysis. χ^2 tests were performed to assess the degree of heterogeneity between trials, and both fixed and random-effects metaregression models fitted.
Results: 17 trials relevant trials were identified. 13 studies, containing a total of 1802 patients and 2199 controls, contained sufficient data for inclusion in meta-analysis of MBL2 genotype and tuberculosis infection. Separate analyses were performed for HIV-positive and negative patients. Overall, no significant association could be demonstrated between MBL2 genotype and tuberculosis infection (OR for AA v AO/OO 0.95, 95% CI 0.77–1.17). 5 studies, containing a total of 436 patients with active tuberculosis and 603 controls, had sufficient data regarding serum MBL levels. In these studies, serum MBL levels were shown to be consistently elevated in the setting of tuberculosis infection, to a degree consistent with the acute phase reaction.
Conclusions: This analysis suggests that neither MBL2 genotype nor MBL serum levels significantly affect susceptibility to tuberculosis infection.

Serious Gram-positive infections: the need for new treatment options (Symposium supported by Astellas)

S301-S304 **Serious Gram-positive infections: the need for new treatment options**

J. Garau, A. MacGowan, D. Nathwani, M. Niederman (Barcelona, ES; Bristol, Dundee, UK; Mineola, US)

Serious Gram-positive infections are becoming increasingly difficult to treat because of the escalating incidence of multidrug-resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA). In order to improve clinical outcomes and to select the most appropriate therapy, it is essential to consider pharmacokinetic (PK) and pharmacodynamic (PD) parameters, such as PD targets and their relation to dosing and the use of PK parameters as measures of exposure. In addition, AUC/MIC ratios should be used to predict clinical efficacy, and the relationship between MICs and dosing requirements should be considered.
In patients with suspected multidrug-resistant infections, prompt initiation of appropriate therapy is an important determinant of mortality. In some European hospitals MRSA strains account for >40% of all *S. aureus* isolates so it is crucial that the chosen therapy provides appropriate coverage for MRSA. Current treatment guidelines for serious Gram-positive infections, such as hospital-acquired pneumonia (HAP) and complicated skin and soft tissue infections (cSSTIs), recommend the use of vancomycin or linezolid for infections caused by MRSA. However, the role of these agents in the treatment of serious Gram-positive infections is still uncertain and there is currently an unmet need for additional agents that are effective against serious Gram-positive infections caused by both methicillin-susceptible *S. aureus* (MSSA) and MRSA.
Telavancin is a rapidly bactericidal lipoglycopeptide with a novel dual mode of action and a broad spectrum of activity against clinically important Gram-positive pathogens, including MRSA. In phase 3 trials, telavancin has demonstrated non-inferiority compared with vancomycin for the treatment of cSSTIs and HAP (including ventilator-associated pneumonia) caused by Gram-positive pathogens, including MSSA and MRSA. These results suggest that telavancin would be a promising addition to current therapeutic options in the management of serious Gram-positive infections caused by MSSA and MRSA.

The medical and public health impact of rapid molecular testing (Symposium supported by Cepheid)

S308-S311 **The medical and public health impact of rapid molecular testing**

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Despite notable advances in recent years, molecular diagnostics applications for infectious disease detection have yet to reach their full potential. Often performed in specialized laboratories far removed from where patient care decisions are made, specimens usually spend more time in transit than in actually being analyzed, and once samples do arrive in the lab, further delays are incurred by obligatory dependence on batch processing.
The chief limitations of current molecular diagnostic approaches are not due to the underlying technology, but rather in how this technology is implemented. Real-time PCR is perfectly capable of generating results within a few minutes. However, the processes leading up to the analytical steps are often unwieldy and performed in batches within a high-complexity laboratory environment. Moreover, the requirement for complex procedures ties the availability of molecular diagnostic test results with the limited availability of highly trained personnel.
In many cases, medical value of a diagnostic result is tied directly to how quickly results can be linked to patient management and/or treatment decisions. Fortunately, technologies for nucleic acid detection are now evolving in the direction of modern clinical chemistry analyzers, the most successful of which allow for random access, high throughput, and STAT testing capability. In addition, by virtue of the simplicity afforded by integrated, automated sample processing, the technology is fully capable of being decentralized in order to minimize the impact of sample transport on turnaround time. This symposium will focus on the "need for speed" in driving maximum medical impact for four different areas relevant to molecular diagnostics – respiratory infections, group B strep prophylaxis, blood cultures positive for staphylococci, and pre-surgical prophylaxis.

Current and future management of fungal infections (Symposium supported by Pfizer)

S312 **Recent results with voriconazole prophylaxis**

C. Kibbler (London, UK)*

Objectives: Despite increasing understanding, the treatment and prevention of invasive fungal infections (IFIs) remains a major challenge. IFI is an important cause of complications and mortality among hospitalised patients, especially among immunocompromised patients in the haematology setting. The potential of voriconazole (VORI) as primary and secondary prophylaxis for IFI has recently been evaluated in clinical trials in patients with haematological disease having undergone allogeneic transplantation.
Methods: In the primary prophylaxis study IMPROVIT (A), patients ⩾12 years were randomised to receive oral VORI or oral itraconazole (ITRA) from the day of transplantation, for 100–180 days; the primary composite endpoint was success of prophylaxis at Day 180 (i.e. survival without developing proven/probable IFI or discontinuing prophylaxis for

>14 days during the first 100 days). In the secondary prophylaxis study VOSIFI (B), patients ⩾18 years with proven/probable IFI in the previous 12 months received VORI 4 mg/kg/12 hours IV or 200 mg/12 hours PO within 48 hours post-conditioning chemotherapy, for 100–150 days; the primary endpoint was the incidence of proven/probable IFI during the subsequent 12 months.

Results: In A, the success of prophylaxis was significantly higher in the VORI group (n = 234) compared with the ITRA group (n = 255) at Days 100 (55% vs 41%; 95% CI: 6%, 24%; p = 0.0007) and 180 (49% vs 35%; 95% CI: 7%, 24%; p = 0.0004). Significantly more patients treated with VORI than ITRA (54% vs 40%; p = 0.0014) had sufficient days of prophylaxis (median: 97 vs 68 days). While the incidence of IFI was low in both arms (VORI: 1.3%, ITRA: 2.4%), 3 patients receiving ITRA developed IFI compared with none on treatment with VORI. In B (n = 42), 3 cases of IFI occurred: 1 recurrent *Candida albicans* candidaemia, 1 recurrent *Scedosporium prolificans* fungaemia (fatal) and 1 new case of zygomycosis, at Day 3, 16, and 66 after transplant, respectively (incidence: 7%). The most common VORI-related adverse events were hepatotoxicity, nausea, headache and hallucinations/visual impairment (<15%). In A, there was no difference in 180-day survival (85% in each treatment group); in B, 11 patients (24%) died (median: 136 days post-transplant) but only 1 from IFI.

Conclusion: Based on the results of these prophylaxis studies, VORI is an effective and safe option for both primary and secondary prevention of IFI after allogeneic transplantation.

Microorganisms as human carcinogens: a list without an end

K317 Micro-organisms as human carcinogens: a list without an end

L. Gissmann (Heidelberg, DE)*

The current estimation of the cancer cases that are related to infectious events varies between 18 and 20% and there is evidence that this number is still growing. Besides *Helicobacter pylori* and some parasites there are mostly viruses for which a causative relation to malignant diseases has been demonstrated. Establishing such a link depends on the epidemiologic profile of the disease and the putative causal infectious agent as well as its biologic properties in experimental cell culture and *in vivo* models. Often one of the classical Koch's postulates (isolation of the putative agent from the affected tissue) cannot be fulfilled and, in some instances, not even traces of the microbe (i.e. its nucleic acid) can consistently be found in the tumor. The reason might be that cancer is the late and rare consequence of a chronic infection where replication of the infectious agent does no longer take place and gene functions are not required for maintenance of the tumor growth. Therefore a combination of observational and experimental strategies has to be applied to collect sufficient evidence and to initiate attempts for preventive measures that target the infectious agent such as development of vaccines. Examples of classical and innovative approaches will be presented.

Reporting β-lactam susceptibility tests on Enterobacteriaceae

S318 Pro – report in accordance with test results

J. Turnidge (North Adelaide, AU)*

The emergence of extended-spectrum β-lactamases (ESBLs) in Enterobacteriaceae (ENTB) in the 1980s began to alter the laboratory approach to β-lactamase detection in Enterobacteriaceae. Prior to that time, results for β-lactams were reported according to the test result, and specific detection of acquired narrow spectrum β-lactamases such as TEM-1 was not sought. With the arrival of ESBLs, detection and confirmation was considered very important, and the default position arose that the presence of an ESBL in a test clinical strain, whatever the MIC of the organism, required the reporting of the organism as resistant to cephalosporins of all types. Many susceptibility testing methods recommended the use of ESBL screening and confirmation tests on a routine basis. However, a number of issues have emerged routine use over the years:

1. The problem of defining an adequate number of substrates to ensure sufficiently sensitive screening.
2. The lack of simple and reliable phenotypic methods to detect ESBLs in species with inducible AmpC β-lactamases.
3. The failure of current ESBL detection methods to provide advice on the interpretation of a positive screening test but a negative confirmation test, especially if the isolates are "susceptible" to extended-spectrum cephalosporins using method-recommended breakpoints.
4. Animal model data showing that the response to treatment of ENTB (measured as *in vivo* killing) is correlated with the MIC of the strain, and not the presence or absence of an ESBL.

A range of recent studies has suggested that failures of treatment with extended-spectrum cephalosporins are likely when strains of ENTB have MICs elevated above the wild-type. Further, application of pharmacokinetic/pharmacodynamic principles to the most widely recommended dosing schedules of cephalosporins have shown that the susceptibility breakpoints recommended by many methods have been too high. Lower breakpoints for injectable cephalosporins are now published by CLSI and EUCAST, with recommendations to optionally conduct specific ESBL tests, for instance if there is an epidemiological need. These newer cephalosporin breakpoints put us in a position where almost all ESBL-producing and plasmid-borne AmpC-producing ENTB will be detected and reported as I or R with the new breakpoints, and specific β-lactamase testing will not be required. This is appropriate for clinical reports whose major purpose is to guide therapy in individual patients.

S319 Con – report in accordance with resistance mechanism

D. Livermore (London, UK)*

It is argued that treatment outcomes, e.g. for ESBL producers, can be predicted from MICs, irrespective of the resistance mechanism. Proponents of this view contend that it is unnecessary for clinical laboratories to edit susceptibility data on the basis of resistance mechanisms and that these should only be sought, if at all, for purposes of epidemiological surveillance.

Several outcome studies support this view (though others do not) and it would have great merit in a perfect world where routine MICs were rapidly and precisely determined for clinical isolates. The reality is very different. MICs, if measured at all in clinical laboratories, are determined, late, on a geometrical scale with four-fold run-to-run variation tolerated for control strains. Often zone diameters are used as a proxy for MICs and, as anyone who has ever run a multicentre disc-based survey discovers, these are prone to disturbingly wide site-to-site variation. In this imperfect reality an ESBL producer with a cefotaxime MIC of 1 mg/L (probably responsive to cefotaxime *in vivo*) cannot be reliably distinguished from one with an MIC of 4 mg/L (probably not responsive) and it is simpler and safer to follow the precautionary approach of seeking the ESBL and, if this is found, reporting the isolate as resistant.

A further advantage of seeking mechanisms is that – using selective primary culture media or chromogenic tests – they can be found by 24-h post specimen, as against 48-h for a precise MIC. This differential, which is only likely to increase with the coming of molecular methods to detect resistance mechanisms, may be critical, allowing the association between early effective therapy and reduced mortality in severely-ill patients.

Antiretroviral therapy

O324 Longitudinal evaluation of lipoprotein-associated phospholipase A2 as a cardiovascular disease-associated biomarker in relation to abacavir therapy

J. Parra-Ruiz, A. Pena-Monje, C. Tomas-Jimenez, V. Guillot, M. Alvarez, J. Lopez-Bueno, F. Garcia-Garcia, J. Hernandez-Quero (Granada, ES)*

Objectives: As there exists still some concern about the potential relationship of Abacavir (ABC) and myocardial infarction (MI), and some authors has postulated that the increase in the risk of MI might be caused because of pro-inflammatory state, as increase and decrease of the risk follows quickly the start and resume of ABC, we decided to longitudinally evaluate Lipoprotein-associated phospholipase A2 (Lp-PLA2), a widely accepted marker of vascular inflammation and cardiovascular disease during ABC therapy in HIV patients due the lack of accuracy of other markers as IL6, IL8, hs-CRP or TNFalfa.
Methods: Eleven consecutive HIV-positive patients starting ABC-containing HAART were sampled at 0, 6 and 12 months after the start of ABC. Eleven HIV-positive patients on ABC sparing HAART were individually matched for other potential cardiovascular disease markers as hypertension, sex, age, smoking status, cholesterol and diabetes, and were sampled as controls.
Results: There were 4 women and 7 men in the cases matched against the same proportion of males/females. Median age was 42 years old (32–54) and 42 (30–54) for men in cases and control, respectively and 40 (35–46) and 40 (33–46) for women in cases and controls respectively. Results are depicted in Table 1. Values of LpPLA2 are expressed in ng/mL.
Mean values for every patient at any given time point were higher than clinical cut points established in 2006 (low risk Lp-PLA2 <200 ng/mL). Interestingly, there was an increase in mean LpPLA2 in all four groups 6 months after the change in HAART, followed by a decrease. At 6 months there were no differences in men regarding ABC, but at 12 months there were an increase in men taking ABC whereas there were a decrease in men without ABC ($p = 0.02$). Women did not show any differences.
Conclusion: There is an increase in Lp-PLA2 during therapy with ABC in men, but because there were no MI during the one year follow-up among the two groups, the mean Lp-PLA2 values are more than 3 times higher than clinical cut points and Lp-PLA2 seems to exhibit a "risk threshold" we cannot rely on LpPLA2 as a cardiovascular marker in our population.

	Mean (range)		
	Start	6 months	12 months
Men			
ABC	614.41 (483.1–812.3)	702.9 (582.3–860.9)	669.92 (497.3–835.94)
No ABC	653.74 (517.3–778.1)	693.6 (615.2–769.5)	620.8 (483.1–764.5)
Women			
ABC	535.7 (450.9–661.6)	548.8 (480.9–670.2)	566.83 (483.8–685.94)
No ABC	644.6 (503.1–763.8)	646.4 (542.3–723.4)	652.72 (472.3–806.6)

O325 Effectiveness of tenofovir-abacavir containing HAART in pretreated HIV-1 infected patients

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Objectives: Although there has been an increase in available antiretroviral for pretreated patients, their high cost and short time since approved make nucleoside reverse transcriptase inhibitors (NRTI) to be the most widely used backbone. However, since pre treated patients usually harbored mutations in reverse transcriptase, frequently tenofovir (TDF) and abacavir (ABC) are the only NRTI available. Since there are no reports about the combination of ABC and TDF, ans some concern about drug-drug interaction, we made a retrospective review to establish the effectiveness of ABC plus TDF as a NRTI backbone of HAART in pretreated patients.
Methods: A single clinic retrospective study including all HIV-1 infected pretreated patients >18 years of age starting ABC-TDF irrespective of their prior HAART. The effectiveness was evaluated in an ITT analysis.
Results: Forty-six patients, 31 men (67%) with a mean age of 43 years (28–65) and median CD4 cell count of 447 (75–1935). Mean HIV-1 viral load was 29400 copies/mL (19–85600). Among the sample 12 patients (26%) had undetectable viral load when starting the combination. Failure, as defined, occurred in 26 patients (57%), with 21 patients (46%) having virological failure. The other 5 patients resumed therapy because side effects to any of the drugs (4 patients) or lost to follow up (1 patient). Among these 5 patients, 3 had undetectable HIV-1 viral load when stopped therapy. Median duration of therapy for non-failure patient was 27 months (11–54) and 12 months (3–33) for patients who resumed therapy. When stratified by viral load at the starting of therapy we found that patients who had undetectable viral load had better chance to keep on therapy than patients who started with detectable viral load 8/12 (66%) vs 12/34 (35%), almost reaching statistical significance ($p = 0.061$).
Conclusion: TDF-ABC backbone should not be routinely used in HIV pre-treated patients. However, if the patient has undetectable viral load, the combination might be used with a high chance of success at 96 weeks follow-up in an ITT analysis.

O326 Optimization of antiretroviral treatment of HIV-patients with viral loads below 1,000 copies/mL based on genotyping data

J. Mier-Mota, L. Lopez-Cortes, P. Viciana, P. Perez-Romero (Seville, ES)*

Objectives: Despite the success of antiretroviral therapy (ART) in the majority of patients, a percentage of patients receiving ART have sustained low-level viremia with viral loads (VL) between 20 and 1,000 copies/mL. The objective of this study was to determine whether the early optimization of the treatment based on the resistance pattern determined in patients with VL below 1,000 copies/ml results in the decrease of the VL to undetectable levels.
Methods: Our laboratory has developed a method for amplifying and sequencing the protease and retrotranscriptase genes from the plasma of patients with VL between 20 and 1,000 copies/mL. In this method HIV-RNA is concentrated using RNA affinity colums and is used as a template for high fidelity RT-PCR. The products are sequenced and mutations determined and analyzed. Using this method we studied HIV patient samples collected from September 2008 to September 2009. Genotyping data were obtained for each patient and ART was modified according to the resistance pattern and previous clinical history.
Results: Genotyping was determined for 37 patients that fullfilled the requirements of sustained low-level viremia. Resistance mutations were detected in 89% of the sequences. The most common mutations in the protease were at positions 10 (40.5%), 71 (16.2%) and 90 (18.9%). The most common mutations in the retrotranscriptase associated with resistance to nucleoside analogs were at positions 67(27.1%), 184 (27.1%) and 215 (37.5%), while the most prevalent mutations associated with resistance to non-nucleoside analogs were at positions 98 (10.4%), 100 (14.6%) and 103 (16.7%).
After genotyping, 17 patients received an optimized treatment regimen. In 15 of these patients (88.2%) the VL became undetectable, in one patient (5.9%) theVL decreased significantly but not to undetectable levels and in one patient (5.9%) the VL was undetermined. Of the 20 patients that did not undergo treatment change, in 4 patients (20%) the VL decreased to undetectable levels, in 9 patients (45%) the VL remained between 20 and 1000 copies/ml, in 3 patients (15%) the VL increased to over 1,000 copies/mL and in 4 patients (20%) the VL was undetermined.
Conclusion: The developed method allows for genotyping of patients with VL between 20 and 1,000 copies/mL. Treatment changes based on these genotyping data have clinical significance since the VL decreased to undetectable levels in the majority of the patients who underwent treatment change.

O327 Lack of correlation between plasma residual viraemia and total HIV-DNA in PBMCs of successfully treated patients

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Objectives: The origin of residual viremia (RV) in HAART-treated HIV-seropositive patients (pts) is unknown. Many investigators interpret RV as the result of ongoing cycles of replication, others as the reactivation of virus from latently infected cells. The aim of the study was to correlate the level of HIV-RNA in plasma and level of HIV-DNA in PBMCs in a cross-sectional analysis.
Methods: 195 HAART-treated pts who achieved virological suppression, as defined by two consecutive plasma HIV-RNA measurements <50 copies/ml, from at least 18 months were enrolled. On the basis of RV values, the pts were subdivided in 4 groups: pts with undetectable plasma RNA level (UL, <1 copy/ml), pts with low level (LL, >1–10 copies/ml), pts with high level (HL, >10–50 copies/ml) and pts with viral blip (VB, >50–400 copies/ml). RV was quantified by an ultra-ultrasensitive method based on a modified Amplicor HIV-1 Monitor 1.5 (Roche Molecular Systems, USA), with a detection limit of 1 copy/ml. To quantify the total proviral HIV-DNA copy number in PBMC, the Real Time TaqMan protocol published by J-P Viard was adapted, with a sensibility of 5 copies/10^6 PBMCs.
Results: 66 (33.8%) pts were UL, 63 (32.3%) were LL, 41 (21%) were HL and 25 (12.8%) were VB. UL pts had highest number of nadir CD4+cells compared to other groups (UL: 360 cells/ml, LL: 315 cells/ml, HL: 279 cells/ml and VB: 305 cells/ul; mean values). Not significant difference was detected in CD4 cell count in the four groups of pts (698, 764, 680 and 691 cell/ul, respectively; mean values). Twenty two patients had undetectable level of proviral DNA in PBMCs (10 UL, 2 LL, 9 HL and 1 VB). The 12 patients with undetectable proviral DNA but detectable residual viremia had a median of 22 copies of HIV-RNA copies/ml (range 12–81 copies/ml). Finally, 11 out of 31 (35.5%) pts with more than 1,000 copies of HIV-DNA/10^6 PBMCs had undetectable level of RV.
Conclusion: A lack of correlation between HIV proviral DNA and residual viremia levels in a cohort of virological long term suppressed pts was demonstrated. This study confirms the complex origin of RV and the need to evaluate the relevance of the impact of the new potent drugs, such as Integrase Inhibitors and CCR5 Inhibitors, on episomal viral DNA, on RV and on the long term treatment success.

O328 Prevalence of secondary drug-resistant mutations to antiretroviral drugs in Iranian HIV-infected patients

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Objective: Several studies have reported increasing number of therapeutic failures with antiretroviral drugs in HIV infected patients. The emergence of viral resistant strains is a major problem for the medical management of infected individuals. In this study we aimed to determine the prevalence of secondary antiretroviral resistance-associated mutations in Iranian HIV infected patients.
Methods: A total of 40 HIV infected patients under antiretroviral drug treatment were enrolled in this study. All of the patients were received antiretroviral treatment for at least 1 year. One protease inhibitor (PI) or one nonnucleoside transcriptase inhibitor (NNRTI) in combination with 2 nucleoside transcriptase inhibitors (NRTI) are considered for antiretroviral therapy.
The HIV pol region including viral protease and reverse transcriptase genes were amplified and sequenced for determining genotype, subtype and antiretroviral resistance-associated mutations.
Results: Sequencing of the samples revealed that 40% of strains belonged to subtype B, 20% subtype A, 35% were A/D and 5% were CRF01-AE recombinants. Drug resistance-associated mutations identified more common in subtype A/D recombinant. Virus samples from 30% of participants showing no drug resistance mutation and 70% of them carried ⩾2 drug resistance mutations. Dual-class drug-resistant virus (NRTI and NNRTI) was present in 22% of participants, and 43% had virus with triple-class drug resistance.
The prevalence of NRTI mutations was 77% with M184V and V118I present in 55% and 48% of samples respectively. The prevalence of NNRTI mutations was 66% which P225H mutations were present in 30% of study specimens. The prevalence of PIs mutations was 44%. Major PIs mutation L90M was seen in 45% and minor protease inhibitor mutation A71V was detected in 50% of samples. The other major PIs mutations were V32I, L33F, M46I, I54V, V86T, and I84V.
The highest frequency of resistance to PIs was related to nelfinavir (60%, high level resistance) and saquinavir (60%, intermediate resistance).
For NNRTI, the frequencies of resistant isolates were 56% to nevirapine, 44% to delavirdine and 44% to efavirenz, and for NRTI was 56%, high level resistance to lamivudine.
Conclusions: Our study showed a high prevalence of secondary resistance mutations in Iranian HIV infected patients. Continued surveillance of resistance patterns is warranted to guide therapeutic approaches as selection of second-line regimens in Iran.

Interferon-gamma release assays for TB diagnosis

O329 Performance of interferon-gamma CSF tests in the diagnosis of TB meningitis in children

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Objectives: We investigated whether a rapid diagnosis of TB meningitis at children can be established by interferon-gamma blood and cerebrospinal fluid tests in routine clinical practice and assess the level of agreement with classic laboratory methods.
Methods: The patients have been separated in two groups: first group, where the diagnosis of TB meningitis has been confirmed by positive culture from CSF and efficacy of treatment; second group with non-TB meningitis. We performed QuantiFERON-TB Gold in Tube (QFT-G) in blood and CSF at all patients.
Results: From a total of 46 children enrolled, 21 cases has been diagnosed with TB meningitis and 25 patient was in the control group; B:F = 18:10; mean of age was 9.75 (age range 6 months to 17 years). The symptoms and physical examination at the admission may suggest TB meningitis: fever, headache, vomiting and neck stiffness at 17 patients, cranial nerve palsies at 2 patients, hemi paresis at 3, altered mental status at 13 and seizures at 6 subjects.
TST was positive at 13 patients in the first group and at 6 in the second. The CSF analyses were suggestive for TB at 16 patients: pleocytosis ⩽300 elements/ml, high CSF protein level and low CSF glucose. All patients had negative smear and only 7 (33.3%) had positive culture. The mean time interval required for QFT-G results was 48 hours and for cultures was 25.6 days. In the first group, the results of QFT-G in blood samples were: 15 (71.4%) subjects had QFT-G positive, 5 (23.8%) had QFT-G negative and one indeterminate (4.7%). In CSF, 16 (76.2%) patients had positive results, 3 (14.3%) had negative and 2 (9.5%) indeterminate. In group of non TB meningitis, 23 (92%) patients had negative results in blood and 2 (8%) positive; in CSF, 19 (76%) subjects had negative results and 6 (24%) indeterminate. The sensitivity of QFT-G in CSF was 84.2% and 75% in blood; specificity 100% in CSF and 92% in blood; the positive predictive value of QFT-G was 100% in CSF and 88.2% in blood; negative predictive value was 86.3% in CSF and 82.1% in blood. The sensitivity of TST was 61.9% and specificity 76%. The sensitivity of the culture from CSF was only 33.3%. The sensitivity and specificity of QFT-G was higher than TST and culture and better in CSF than in blood.
Conclusions: The determination of γ-interferon in serum and CSF is useful diagnostic marker of tuberculosis who could improve the management of TB meningitis, by a rapid diagnosis and an early treatment initiation.

O330 Sensitivity, specificity and inter-test agreement of interferon-gamma release assays for the diagnosis of latent tuberculosis infection in HIV-infected individuals with advanced immunodeficiency

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Objective: Treatment of latent tuberculosis (LTBI) in HIV+ patients reduces the incidence of TB. Interferon-gamma release assays (IGRAs) may be more specific in diagnosing LTBI than the tuberculin skin test, but their sensitivity at lower CD4 counts has not been established. We studied the effect of CD4 count on IGRA results.
Methods: T-SPOT.TB® ELISPOT assays, incorporating ESAT-6 and CFP-10, and Quantiferon 3G® ELISA assays, incorporating ESAT-6, CFP-10 and TB7.7, were performed in HIV+ patients with CD4 counts $<250\times10^6$/L (n = 72, median CD4 count = 124×10^6/L) and HIV+ controls with CD4 $>250\times10^6$/L (n = 160, median CD4 count = 460×10^6/L). Active TB was excluded in all patients. ELISA and ELISPOT responses were related to patient characteristics.
Results: ELISA responses were positive in 21% (n = 15) of patients with CD4 $< 250\times10^6$/L and 31% (n = 49) of those with CD4 $> 250\times10^6$/L, (difference of 10%, 95% CI –2–22%, p = 0.15). ELISPOT responses were positive among 8% (n = 5) of patients with CD4 $< 250\times10^6$/L and 15% (n = 22) of those with CD4 $>250\times10^6$/L, (difference of 7%, 95% CI –2–16%, p = 0.13). However; when multivariate regression analysis was used to adjust for LTBI risk factors such as origin from countries of high TB prevalence, ELISA and ELISPOT results were independent of CD4 count.
Migration from countries with a high prevalence of TB (COHP) was associated with positive results on multivariate analysis: in those from COHP the OR for positive ELISA was 2.7 (95% CI 1.5–5), p = 0.03; and OR for positive ELISPOT was 4.0 (95% CI 1.6–10), p = 0.003. Injection drug use, incarceration and homelessness were not associated with positive results. Significantly, 17% (7/41) of individuals with no risk factor for LTBI had a positive ELISA, and 5% (2/41) had a positive ELISPOT.
30% (70/232) of patients had one or more positive IGRA result, of whom only 32% (21/70) were positive on both tests. Agreement between the two IGRAs was moderate, Cohen's kappa = 0.37. There was no association between inter-test agreement and CD4 count. Origin from a COHP was associated with a higher level of agreement between positive tests.
Conclusions: ELISA and ELISPOT responses were independent of CD4 count. Disagreement between the tests is frequent, and not related to CD4 count. Discordant results are more common in patients with lower pre-test probability of LTBI and, given the high rate of positive results in those with no risk factor for LTBI, may represent false positive results.

O331 Clinical experience with Tspot.TB™ for diagnosis of tuberculosis in HIV-negative and HIV-positive patients

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Aims: IFN-γ release assays (IGRA) had principally been developed for the diagnosis of latent tuberculosis infection (LTBI) and appear more sensitive and specific than than tuberculin skin testing. IGRAs may also offer an attractive option for assisting in diagnosis of active TB. Several studies have been published comparing IGRAs with conventional diagnosis. As part of on-going assessment of TB diagnostic approaches we assessed the reliability of IGRA within our cohort at the regional Tropical and Infectious Diseases Unit (TIDU).
Methods: All patients who underwent Tspot TB testing within the TIDU, since testing was introduced in Jan 2008, were included. Comparison was made with *M. tuberculosis* culture and with a final diagnosis of TB (the administration of a full course of TB therapy). The comparison was also performed separately for both HIV+ and HIV– patients.
Results: 73 Tspot.TB tests were performed on 72 patients; 32% of patients (n = 23) were HIV+, 8 were not HIV tested. 76% of patients (n = 55) had mycobacterial culture results available (36%, n = 20 HIV+). 94% of patients (n = 68) had data on the administration of TB therapy; 35% (n = 24) received TB therapy (7 HIV+). The kappa coefficient for agreement between Tspot TB and culture of *M. tuberculosis* for all patients was 0.42 (standard error (SE) 0.13); the kappa value for receipt of TB therapy was 0.47 (SE 0.12). For HIV+ patients (n = 23) the kappa value compared with culture was 0.38 (SE 0.22) and for TB therapy was 0.27 (SE 0.2). For HIV- and untested patients (n = 46) the kappa value for culture was 0.43 (SE 0.16) and the kappa value for TB therapy was 0.56 (SE 0.15). The sensitivity of Tspot.TB in all patients compared with culture was 69.2% (95% CI 57–81.4), specificity 78.6% (95% CI 67.8–89.4), positive predictive value 50% (95% CI 36.8–63.2) and negative predictive value 89.2% (95% CI 81–97.4). Compared with receipt of TB therapy in all patients the sensitivity of Tspot TB was 58.3% (95% CI 45.3–71.3) and specificity 86.7% (95% CI 77.7–95.7). Further analysis will be presented.
Conclusion: The performance of IGRA (Tspot TB) in our everyday clinical use, compared with other published studies is poorer for both HIV positive and negative patients. This indicates the need for further prospective studies to be undertaken before recommending IGRA as a diagnostic tool in active TB.

O332 Usefulness of interferon-gamma release assays for latent tuberculosis screening in patients candidate for TNF-α therapy

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Objectives: Reactivation of *Mycobacterium tuberculosis* infection is a major complication of anti-tumor necrosis factor (TNF-α treatment. Thus, screening for latent tuberculosis infection (LTBI) is mandatory before starting anti-TNF therapy. The TST has 2 main disadvantages: the low specificity with false positive results in BCG vaccinated subjects and lower sensitivity in presence of immunosuppressive therapy resulting in false negative results. Diagnosis of LTBI may benefit from new interferon-gamma release assays (IGRA). The aim of this study was to investigate the performance of QuantiFERON-TB Gold In Tube (QFT-GIT) assay and its agreement with TST in the screening of LTBI in patients with an immune-mediated inflammatory diseases candidate for anti-TNF therapy.
Methods: We consecutively enrolled 215 patients affected by rheumatoid arthritis (n = 77), psoriatic arthritis (n = 38), psoriasis (n = 64), ankylosing spondylitis (n = 8), Crohn's disease (n = 22), and Behcet's disease (n = 6) were enrolled. Screening included: clinical evaluation, chest X-ray, TST and QFT-GIT. 106 patients were on corticosteroid and/or immunosuppressive drugs and 20 were BCG-vaccinated.
Results: Of the 215 patients, 34 (15.8%) had a positive, 152 (70.6%) a negative, and 29 (13.4%) had an indeterminate QFT-GIT result. In 122 (65.5%) patients the TST and QFT-GIT were both negative, in 30 (16%) patients both the tests were positive, while 34 (18%) had discrepant results between TST and QFT-GIT. Agreement between two tests was 81.7% (k = 0.53). A diagnosis of LTBI was considered in 38 cases (17.6%). By univariate analysis, we observed an association between BCG vaccination and discordant QFT-GIT-/TST+ (OR = 6; 95% CI: 2.3–37.1; $p < 0.001$) and between the immunosuppressive therapy and an indeterminate QFT-GIT tests (OR = 2.79; 95% CI: 1.06–7.6; $p < 0.02$). No association between the immunosuppressive therapy and discordant QFT-GIT+/TST– (OR = 0.16; 95% CI: 0.01–1.8; $p < 0.09$) was found.
Conclusion: Our results show that QFT-GIT may be helpful for screening LTBI in patients candidates for anti-TNF-α therapy not only to confirm positive TST results but also to discriminate false-negative TST results as a consequence of previous long-term immunosuppressive treatments. The performance of QFT-GIT seems to not be affected by immunosuppression in our patients. IGRA appears to offer a better chance than TST for monitoring TB infection during anti-TNF-α therapy.

O333 Diagnosis of tuberculosis infection in patients awaiting transplantation

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Objectives: To compare the tuberculin skin test (TST) with the QuantiFERON® TB Gold-in Tube (QFT-GIT) for the diagnosis of tuberculosis infection (TBI) in patients prior to transplantation.
Methods: A prospective, cross-sectional study of the patients evaluated for liver and haematopoietic progenitor transplantation in a teaching hospital in Spain, from July 2008 to September 2009. Simultaneous QFT-GIT and two steps TST were performed.
Results: 92 patients were screened for TBI, 69 (75%) candidates to liver transplantation (LT) and 23 (25%) to haematopoietic progenitor transplantation (HPT). Sixty (65.2%) were men, mean age of 55 years. Fifty two (56.2%) presented some risk factor for TBI and 23 (25%) had BCG-vaccination. In the LT group, 67 (97.1%) patients were diagnosed with cirrhosis with a mean MELD score of 13.8. In the HPT group, 21 (91.3%) patients had received chemotherapy, at least, in the previous 6 months. Thirty eight (41.3%) patients had a positive TST (35 with the 1st TST and only 3 with the 2nd one), and also 38 (41.3%) had a positive QFT-GIT. Among LT patients, there were 31 (44.9%) positive TST and 31 (44.9%) positive QFT-GIT results, and among HPT patients 7 (30.4%) positive TST and 7 (30.4%) positive QFT-GIT results. In the LT group, a MELD score >18 (OR 0.09, CI95% 0.02–0.55; $p = 0.01$) and albumin <30 (OR 0.23, CI95% 0.07–0.82; $p = 0.02$) were associated with a lower likelihood of positive TST but not with QFT-GIT results. Discordant results were observed in 11 QFT-GIT+/TST− patients and in 11 QFT-GIT−/TST+. QFT1-GIT+/TST− results were associated with MELD score >18 (9.8, CI95% 1.6–62; $p = 0.02$). There were only 3 (3.3%) indeterminate QFT-GIT results.
Conclusions: QFT-GIT and TST are both feasible tests for diagnosing TBI in patients considered for LT and HPT. Higher MELD score and hypoalbuminemia were associated with a lower likelihood of positive TST, but not with QFT-GIT.

Molecular virology

O334 Epstein–Barr virus gene expression patterns in paediatric liver transplant recipients

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Objectives: Determination of EBV gene expression patterns in association with EBV DNA load monitoring might be more informative and effective tool in monitoring EBV infection in immunosuppressed patients, allowing earlier detection of EBV related lymphoproliferations and therapeutic intervention in time. The aim of this study was to analyse latent and lytic EBV transcript levels, in relation to EBV DNA load in peripheral blood lymphocytes in immunosuppressed paediatric patients after liver transplantation (LTx).
Methods: Thirty patients after LTx were included in this study. Mean±SD age at LTx was 24.1±30 months. Mean follow-up was 36.2 months (range 4.7–122.8 months). Multiple blood samples prospectively collected at different time points during post-transplant checkups were used for EBV load measurement and viral gene expression analysis. Quantitative analysis of latent (EBNA1, EBNA2, LMP1, LMP2) and lytic (BZLF1) gene expression was performed in 75 samples by two-step RT real-time PCR. The B95−8 cell line was used as a calibrator, and the expression level of EBV genes was normalized to the expression levels of the endogenous control (HMBS gene). Simultaneously EBV DNA load was monitored using quantitative real-time PCR method.
Results: In children after LTx several distinct patterns of EBV gene expression were identified. Majority of samples (53/75, 71%) showed latency 2 profile (LMP1±LMP2 expression), in 10 samples (13%) latency 3 was found (EBNA2 + other latent transcripts), whereas latency 0 (LMP2 only expression) was detected in 8 samples (11%). Additionally, in 4 samples from 4 children lytic infection (BZLF1 expression) was identified. In three cases it was accompanied by a simultaneous presence of latent transcripts. In most children (12/16) in whom multiple blood samples were analysed EBV gene expression patterns varied over time. Similarly, expression level of particular EBV genes varied over time up to 5-log fold in individual patients. EBV latency pattern was not associated with the EBV DNA level. Similarly, the appearance of lytic infection (detectable BZLF1 expression) did not lead to a significant increase of EBV copy number. However, viral load correlated significantly ($p < 0.05$) with the LMP1 and LMP2 expression levels (both $r = 0.29$), but not with EBNA1 or EBNA2 transcript levels.
Conclusion: The activity of LMP1 and LMP2 genes may influence EBV DNA load in immunosuppressed paediatric patients.

O335 Prevalence of papillomavirus in HIV-positive patients

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Objective: The aim of the present study was to determine the prevalence and genotype distribution of concurrent anogenital human papillomavirus (HPV) infection in male patients infected with the human immunodeficiency virus (HIV), using polymerase chain reaction and reverse hybridization.
Methods: HPV testing was performed on anal cytology specimens collected from 65 HIV-seropositive men from January to October 2009. Results of de anal cytology also were collected.
Anal samples were collected by a trained nurse and cytological specimens were prepared using a liquid based collection method (ThinPrep).
The samples were loaded onto the EasyMAG system (bioMérieux, Durham, NC) and DNA extractions were made according to the manufacturer's protocol. The genotyping was performed with a new technological platform using a low density Microarray CLART® Papillomavirus 2 (Genomica, Spain). All samples were analyzed for the presence of the following HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85 and 89.
Results: HPV DNA was detected in 58 (89.2% of samples). The most frequently detected HPV types were HPV 53 (29.2% of the positive samples), HPV 16 (24.6%), HPV 33 (24.6%), HPV 51 (23%), HPV 58 (21.5%), HPV 59 (18.5%) and HPV 68 (16.9%). We found only seven patients with one genotype infection of HPVs.
Twenty nine patients (44.6%) with normal anal cytology had high and low risk HPV. In six patients we found high risk HPV and abnormal cytology [3 patients had low-grade squamous intraepithelial lesion (LSIL), 2 patients with grade I anal intraepithelial neoplasia (AIN-I) and 1 patient with atypical squamous cells of undetermined significance (ASCUS)].
Conclusions: A wide variety of HPV genotypes was detected, and co-infection with multiple genotypes was common in ours patients.
HPV screening in HIV-positive patients even in cases of normal anal cytology can be useful for detecting those patient with risk to develop cancer.

O336 Natural polymorphism of UL23 thymidine kinase and UL30 DNA polymerase among herpes simplex virus type 1 and 2 strains

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Objectives: Genotypic detection of herpes simplex virus (HSV) resistance to antivirals is based on thymidine kinase (UL23) and DNA polymerase (UL30) gene sequencing. The interpretation of results requires distinguishing resistance mutations from natural interstrain sequence variations. The objective of this work was to assess extensively the natural polymorphism of pUL23 and pUL30 among HSV strains.
Methods: Three laboratory strains (KOS, G-HSV-2, MH2) and 54 clinical isolates (27 HSV-1 and 27 HSV-2) were studied. Forty strains

were collected from patients who had not received any previous anti-HSV treatment, and 14 strains exhibited acyclovir and foscarnet phenotypic susceptibility using a plaque reduction assay. The entire open reading frame of UL23 and UL30 genes was sequenced. Nucleotide and amino acid sequences were compared with that of reference strains from Genbank: accession numbers X14112 (HSV-1 strain 17) and Z86099 (HSV2 strain HG52).

Results: The interstrain identity of UL23 gene ranged from 99.3% to 100% at the nucleotide level in the 57 HSV strains investigated. There were 19 and 11 variant nucleotides for HSV-1 and HSV-2 strains, respectively. Overall, 19 amino acid changes were identified for HSV-1 strains and 7 amino acid changes for HSV-2 strains (that is, 5.1% and 1.9% of the total codons of the protein, respectively). The analysis of UL30 gene showed >99.5% interstrain identity among all HSV strains. Surprisingly, in HSV-1 strains, 113 variant nucleotides were evidenced, of which 72% produced silent mutations, whereas in HSV-2 strains, only 28 were evidenced, of which 32% produced silent mutations. Thirty and 18 amino acid changes distributed across UL30 DNA polymerase were described for HSV-1 and HSV-2 strains, respectively, corresponding to 2.4% and 1.3% of the total codons of the protein. Of note, one HSV-2 isolate harboured a deletion at codons 1106–1111 in the C-terminal region of pUL30. For both viral proteins, 71 out of 74 amino acid changes identified lied within nonconserved regions.

Conclusion: Our results show that UL23 thymidine kinase and UL30 DNA polymerase are highly conserved among HSV strains, with a weaker variability for HSV-2 strains. Beside previously described mutations, a number of previously undescribed natural variations were identified. This work provides the natural polymorphism map of pUL23 and pUL30 among both HSV-1 and HSV-2 strains that will be helpful for HSV genotypic antiviral resistance testing.

O337 Detection of human bocavirus and human metapneumovirus by real-time PCR in children with respiratory tract infections during four successive winter periods in Belgium

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Objectives: To examine the prevalence of two recently discovered viruses, human metapneumovirus (hMPV) and human bocavirus (hBoV) in respiratory specimens of a pediatric population during 4 successive winter periods (2005–2008) by real-time PCR.

Methods: Nucleic acids were extracted from nasopharyngeal aspirates with a Nuclisens easyMAG platform (Biomérieux). A suspension of Phocine Herpes virus and Phocine distemper virus was added as an extraction/inhibition control to each sample before extraction. Amplifications were performed on a Lightcycler 480 (Roche). For hMPV a separate cDNA synthesis step was included before amplification. The hMPV primers and probe sequence were from Maertzdorf et al.(2004) JCM 42, 981–6. These target the most conserved regions of the nucleoprotein gene of the virus. The hBoV primers and probe sequence target the most conserved regions of the non structural sequence of the virus. Our study included 234 nasopharyngeal samples collected from paediatric patients with symptoms of respiratory tract disease without known cause, collected on UZA between 2005 and 2008.

Results: Only samples with a satisfactory amplification of the extraction/inhibition control were taken into consideration. Twenty-six of 230 samples (11%) were found positive for hMPV, 42 of 226 samples (18%) were found positive for hBoV and 7 of 219 samples (3%) were found positive for both viruses. Both viruses were found during the 4 successive winterperiods, with an incidence ranging from 6% in 2006 to 15% in 2007 for hMPV and from 9% in 2006 to 29% in 2005 for hBoV. When looking at the age distribution, hMPV was found with a prevalence of 9% in children under 1 year, 19% in children between 1 and 2 years, both 14% in children between 2 and 3 and between 3 and 4 and 7% in children older than 4. For hBoV, the prevalence was 13% in children under 1 year, 32% in children between 1 and 2 years, 7% in children between 2 and 3, 38% in children between 3 and 4 and 11.6% in children older than 4. For both viruses, there was no clear pattern of seasonal distribution.

Conclusions: Both hMPV and hBoV were found in each of the 4 successive winterperiods examined between 2005 and 2008 in Belgium in a significant number of children presenting with respiratory tract infections. Although prevalent in all age categories, higher prevalences were found for both viruses in children under 4 years of age. For both viruses, there was no clear pattern of seasonal distribution.

O338 Phylogenetic analysis of human bocavirus isolated from children with acute respiratory illness and gastroenteritis in Iran

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Objectives: Human Bocavirus (hBoV) was first discovered in 2005. Based on phylogenetic analysis and genomic organization, hBoV has been classified to Parvoviridae family. hBoV is prevalent in children worldwide and seems to be associated with respiratory tract illness and acute gastroenteritis. One to three slightly different genetic lineages have been reported among hBoV isolated worldwide. The aim of present study is to determine the prevalence of hBoV and analysis of hBoV phylogeny in children suffering from acute respiratory tract illness and acute gastroenteritis in NRITLD, Iran.

Methods: Clinical samples were collected from 180 patients (⩽17y/o) including 50 exacerbated asthma cases, 83 cases with respiratory tract illness and 47 gastroenteritis cases between 2006 and 2008. The specimens were immediately transported in cold box to the virology laboratory at Virology Research Center, treated and stored at −70ºC until use. For screening, nested PCR were performed using primers in NS-1 coding region. 10 positive samples were subjected to sequencing for VP1/VP2 gene junction. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1.

Results: The hBoV was detected in a range of 6% to 12.8% in different clinical presentations. Most hBoVs were detected in children less than 3 years of age and in cold seasons. Statistical differences were observed in age group and seasonal distribution of hBoV in asthma exacerbation cases (P = 0.031) and gastroenteritis (P = 0.024), respectively. However, there was high nucleotide identity (99.18%-100%) between all hBoV sequences, the phylogenetic analysis resulted in 3 genetic groups (Figure 1). 14 of the 864 nucleotide positions were variable, when compared to Swedish reference strain (accession number NC_007455), with 10 transitions (71.4%) and 4 transversions (28.67%). At the amino acid level, there was 98.46–100% sequence identity among isolates and only two changes were found at codon 152 (S → T) and codon 207 (V → M).

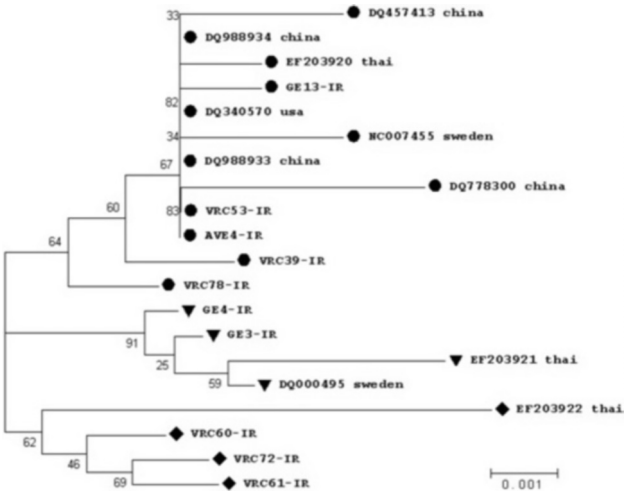

Figure 1.

Conclusion: This study is the first research of phylogenetic analysis of hBoV isolated from children with acute respiratory tract illness and gastroenteritis in Iran. Phylogenetic analysis based on VP1/ VP2 gene junction showed the existence of 3 different genetic groups among hBoV isolates that co-circulate in Iranian population. hBoV from Iran was distributed among all three groups and showed that hBoV is highly conserved and there is no specific-geographical variation.

Community-acquired MRSA and MSSA

O339 Clonal composition and antimicrobial resistance of Panton-Valentine leukocidin-positive *Staphylococcus aureus* in Wales

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Objectives: Panton-Valentine Leukocidin (PVL)-positive *S. aureus* typically cause skin/soft-tissue abscesses, haemorrhagic pneumonia and necrotising fasciitis. Wide dissemination of PVL-positive CA-MRSA clones, i.e. USA300 and USA400, requires continuous surveillance. We investigated the molecular epidemiology and antibiotic resistance of PVL-positive *S. aureus* obtained at two institutions in Wales.
Methods: Two cohorts of PVL-positive *S. aureus* were studied: i. 19/560 (3.4%) consecutive wound isolates from NPHS Microbiology Swansea PVL-positive by PCR (ABMU); ii. PVL-positive *S. aureus* strains submitted to Specialist Antimicrobial Chemotherapy Unit, NPHS Microbiology Cardiff (SACU; n = 61), using mecA, PVL, and arginine catabolic mobile element (ACME) PCR, pulsed-field gel electrophoresis (PFGE), spa-, and SCCmec-typing. Susceptibility testing was performed using BD Phoenix.
Results: mecA prevalence was significantly different between ABMU 2/19 (10.5%) and SACU 35/61 (57.4%) strains ($p < 0.001$). ABMU MRSA strains exhibited t019-MRSA-IVd and t437-MRSA-V genotypes. The majority of ABMU strains were diverse MSSA containing 3 strains each of spa-types t021 and t314 (15.8%) and the new spa-type t4791. The SACU cohort comprised a predominant clone (n = 16; 26.2%) t008-MRSA-IVa, confirmed as USA300–0114 by PFGE and presence of ACME. Other clones included: t044-MRSA-IVc (n = 5; 8.2%); t002-MRSA-IVc (n = 3; 4.9%) and t127-MRSA-IVa (n = 2; 3.3%). Two new spa-types were encountered (1 MRSA, 1 MSSA, assignment pending). Resistant strains (n = 80): oxacillin 45%, gentamicin 5%, tobramycin 36%, erythromycin 28%, clindamycin 1%, tetracycline 18%, trimethoprim 64%, TMP-SMZ 18%, ciprofloxacin 5%, fusidic acid 10%. D-test revealed 36% inducible clindamycin resistant (CR), 5% constitutive CR, and 59% clindamycin sensitive strains in 22 erythromycin resistant strains. Significant differences were seen for SACU/ABMU cohort strains for oxacillin 57%/5%, gentamicin 2%/16%, and tetracycline 10%/42% ($p < 0.05$).
Conclusions: There was a striking difference of antibiotic resistance and clonal composition of the two cohorts. USA300–0114 was the most prevalent clone in the SACU cohort and other globally distributed clones were also represented. None of these clones were found in the unselected ABMU cohort. While molecular epidemiologic analysis of strains submitted to referral units provides valuable information, this needs confirmation by investigation of unselected isolates from the same area.

O340 Frequency of PVL-positive community-associated MRSA and livestock-associated MRSA among patients in Belgian acute-care hospitals

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Objectives: Healthcare-associated strains of methicillin-resistant *Staphylococcus aureus* (HA-MRSA) are endemic in Belgian hospitals for two decades. Recently, PVL-positive community-associated MRSA (CA-MRSA) and livestock-associated MRSA ST398 strains (LA-MRSA) have been identified in Belgium. We determined the relative frequency of MRSA strains by genotype in patients in acute-care hospitals as part of an ongoing surveillance program since 1995.
Methods: All hospitals were invited to collect three consecutive, non-duplicate MRSA isolates from hospitalised patients in 2005 and 2008 (five isolates in 2003). MRSA were confirmed by PCR for nuc and mecA genes and genotyped by spa- and SCCmec-type. MLST was performed on a subset of strains (n = 20).
Results: Of MRSA isolates from 315 patients in 109 hospitals, 53% were detected on admission or ⩽48 hours after admission, compared to 36% in 2005 and 2003. Five PVL-positive strains (1.6%) of ST80-SCCmec IV (n = 4) and ST5-SCCmec IV (n = 1) were detected. PVL-positive MRSA represented 1.2% and 0.2% of MRSA from hospitalised patients in surveys of 2005 and 2003, respectively. Two patients with LA-MRSA ST398 (0.6%), showing spa-type t011, were identified. LA-MRSA isolates were found in 0.9% and 0.4% of MRSA in 2005 and 2003, respectively.
Conclusion: The proportion of patients with MRSA that are imported in hospitals was over fifty percent in Belgium in 2008. However, LA-MRSA and PVL-positive MRSA strains only represent a small proportion (<3%) of the burden of MRSA strains in this patient population in 2008. The frequency of PVL-positive MRSA and LA-MRSA has remained stable since their first detection in 2003.

Table 1: Distribution of MRSA during surveys in 2003, 2005 and 2008

2003 (n = 518)	2005 (n = 327)	2008 (n = 315)
HA-MRSA		
B2-ST45-SCC*mec* IV: 48.5%	ST45-SCC*mec* IV: 47.1%	ST45-SCC*mec* IV: 39.7%
A20-ST8-SCC*mec* IV: 18.7%	ST8-SCC*mec* IV: 22.6%	ST8-SCC*mec* IV: 30.5%
C1-ST5-SCC*mec* II: 13%	ST5-SCCmec II: 12.5%	ST5-SCCmec II: 12.1%
G10-ST5-SCC*mec* II: 5.2%	ST5-SCCmec IV: 4.3%	ST5-SCCmec IV: 6.3%
C3-ST5-SCC*mec* IV: 2.3%	ST22-SCC*mec* IV: 3.7%	
L1-ST22-SCC*mec* IV: 1.9%		
Total: 79.1%	Total: 90.2%	Total: 88.6%
PVL-positive CA-MRSA		
J-ST30-SCC*mec* IV (n = 1)	ST80-SCC*mec* IV (n = 2)	ST80-SCC*mec* IV (n = 4)
	ST30-SCC*mec* IV (n = 2)	ST5-SCC*mec* IV (n = 1)
⩽48 h (n = 1)	⩽48 h (n = 2), no data (n = 2)	⩽48 h (n = 3), >48 h (n = 2)
Total: 0.2%	Total: 1.2%	Total: 1.6%
LA-MRSA		
ST398 (n = 2)	ST398 (n = 3)	ST398 (n = 2)
⩽48 h (n = 1), >48 h (n = 1)	⩽48 h (n = 1), >48 h (n = 2)	⩽48 h (n = 1), >48 h (n = 1)
Total: 0.4%	Total: 0.9%	Total: 0.6%

O341 Association of PVL with staphylococcal pyogenic skin infections

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Objectives: Whilst the role of PVL in staphylococcal disease remains contentious, the association of PVL-positive *S. aureus* (PVL-SA) with recurrent skin and soft tissue infections is compelling. Following the introduction of initiatives for enhanced ascertainment of PVL-related disease in England, we sought to investigate the clinical and epidemiological features of pyogenic skin infections cause by PVL-positive versus PVL-negative *S. aureus*.
Methods: Isolates of *S. aureus* submitted referred to the national *Staphylococcus* Reference Unit (SRU) recovered from pyogenic skin infections (boils, abscesses, carbuncles etc) from patients throughout England, Wales and Northern Ireland during 2008 were included for study. Isolates were characterised by toxin gene profiling (including PVL), mecA testing and DNA fingerprinting by pulsed-field gel electrophoresis. Patient demographic data and clinical features including the site of the lesion(s) and recurrence of infection were analysed.
Results: During 2008, a total of 1230 isolates of *S. aureus* from pyogenic skin infections were submitted to the SRU for characterisation. Of these, 835 were MSSA and 395 were MRSA (68 and 32% respectively). Patients were aged 0 to 95y (median 30y); the male:female ratio was 1:1.
A total of 800 (65%) isolates were PVL-positive; the majority of which (523; 65%) were MSSA. Comparison of cases according to PVL status showed PVL-SA were recovered from younger cohorts (median 26y) and were commonly associated with buttock, thigh and/or axilla lesions (50% cases). In contrast, PVL-negative SA were associated with older

individuals (median 38y) with lesions from a wide range of body sites, the commonest sites being breast or back (11% each). Recurrent infections were more apparent among the PVL group (30 vs 20% cases). A multiplicity of strains/lineages was identified. Of 10 different clones of PVL-MRSA identified, 3 lineages (USA300, European and South West Pacific clones) predominated.
Conclusion: PVL-SA from pyogenic skin infections predominantly occurred in younger individuals, most commonly affecting the buttocks and/or axillae; a third of infections were recurrent. These data have important implications for recognising, diagnosing and managing such infections in the community.

O342 *Staphylococcus aureus* skin and mucosa infections in primary healthcare in Denmark: a 12-year population-based study

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Objectives: A rise in community onset *S. aureus* infections has been observed in several European countries. The objective of this study was to ascertain trends of *S. aureus* infections in primary health care in Denmark.
Methods: We conducted the study within the North Denmark Region (pop. 495,000 inhabitants) 1996 though 2008. We retrieved information on bacteriological specimens collected from skin and mucosal surfaces by general practitioners and practicing specialists. We further obtained data on redeemed dicloxacillin prescriptions which is the preferred anti-staphylococcal penicillin in Denmark. Our unit of observation was a specimen, *S. aureus* isolate or prescription with no similar record within the previous year. According to this definition we limited the study to the years 1997–2008. By use of direct standardization, we computed annual age- and gender standardized rates of bacteriological specimens, *S. aureus* isolates and prescriptions of dicloxacillin per 100,000 person-years (pyr).
Results: A total of 108.758 specimens were obtained of which 42.778 (39%) yielded *S. aureus*. The rate of specimens doubled during the study period reaching 2399 per 100,000 pyr in 2008. The rate of *S. aureus* isolates increased until 2003 and remained fairly stable thereafter (842 per 100,000 pyr in 2008). Among children 0–14 years old, the rate of *S. aureus* isolates increased steeply from 1997 to 2002 followed by a gradual decrease thereafter (peak 145 per 100,000 pyr in 2002). The most prominent trend observed 1997 to 2002 was a 7-fold rise in rate of specimens from impetigo patients (peak rate 217 per 100,000 person-years in 2002). After 2002, the falling rate of impetigo specimens was counterbalanced by an increased number of specimens from wounds in adult and elderly patients. A total of 156.462 dicloxacillin prescriptions were identified, and the rate of prescriptions increased 2.5 times during the study period reaching a level of 3700 per 100,000 pyr in 2007–2008.
Conclusion: The rising incidence of bacteriological cultures and anti-staphylococcal antibiotic prescriptions indicate an increasing attention to *S. aureus* infections in primary health care in Denmark. National campaigns for prudent use of antibiotics may have been an incentive to perform more bacteriological cultures. Impetigo, especially among children, contributed to the rising trend but in recent years there has been a further increase of *S. aureus* isolates from other sources and age groups.

O343 Concomitant prevalence of community- and healthcare-associated MRSA among residents and staff of long-term care facilities in northern Israel

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Background: Long-term care facilities (LTCFs) are important reservoirs of MRSA. We determined the prevalence and molecular characteristics of MRSA among residents and staff of LTCFs in northern Israel.
Methods: Nasal swabs were obtained from residents and staff at 6 LTCFs during February-May 2009. MRSA was identified via growth on CHROMagar™ MRSA plates, and coagulase production. Definitive identification and antibiotic susceptibilities were determined by VITEK® 2. Genetic relatedness of strains was determined by spa-typing, multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE). SCCmec typing was performed via multiplex PCR, and the Panton-Valentine leukocidin (pvl) gene was tested for by PCR.
Results: A total of 191 residents (30% male, median age 85) and 132 staff members (20% male, median age 49) were screened. MRSA was isolated in 14% of residents and in 11% of staff members. The major MRSA strain carried among residents (24/27 isolates, 89%) in 5 of the 6 institutions was a healthcare-associated (HA-) MRSA strain: ST5, t002, SCCmec type II. This HA-MRSA strain was also carried among staff (5/14 isolates, 36%) in 2 of these institutions. HA-MRSA (ST5, t002, un-typable SCCmec) was found as well among staff (2/14 isolates, 14%) in an additional institution in which no residents were MRSA carriers. An additional MRSA strain prevalent among staff (3 institutions, 6/14 isolates, 43%) was a community-associated (CA-) MRSA strain: ST8, t008, with an un-typable SCCmec in 5 of 6 isolates grown. This CA-MRSA strain was detected in only one resident (1/27 isolates, 4%), from an institution in which this strain was found among 4 of its staff members (Table). All MRSA isolates lacked the pvl gene.
Conclusions: MRSA is highly prevalent among residents and staff in LTCFs in northern Israel. Contrary to a priori assumptions, molecular analysis revealed little connection between MRSA among staff and that among residents, arguing against transmission between them. The MRSA strain ST8, t008, with a CA-MRSA phenotype, was prevalent among staff but rare among patients.

Table MRSA details from long-term care facilities in Northern Israel

Status	No.	Screened		Antibiotic susceptibility profile	MRSA molecular characterization		
		No.	Positive MRSA (%)		Spa type	MLST	SCC mec
Facility A							
Residents	15	15	3 (20)	3 $GEN^{S}CIP^{R}CLI^{R}$ ERY^{R}	13 t002	ST5	II
Staff	9	9	0 (0)				
Facility B							
Residents	11	11	2 (18)	2 $GEN^{S/R}CIP^{R}CLI^{R}ERY^{R}$	2 t002	ST5	II
Staff	9	9	0 (0)				
Facility C							
Residents	18	17	2 (12)	2 $GEN^{S}CIP^{R}CLI^{R}ERY^{R}$	1 t2487	–	II
					1 t002	ST5	
Staff	11	8	3 (38)	2 $GEN^{S}CIP^{R}CLI^{R}ERY^{R}$	2 t002	ST5	II
				1 $GEN^{S}CIP^{R}CLI^{R}ERY^{R}$	1 t008	ST8	Un-typable
Facility D							
Residents	12	12	0 (0)				
Staff	14	14	2 (14)	2 $GEN^{R}CIP^{R}CLI^{S}ERY^{S}$	2 t002	ST5	Un-typable
Facility E							
Residents	11	11	7 (64)	6 $GEN^{S}CIP^{R}CLI^{I/R}ERY^{R}$	6 t002	5 ST5, 1 ST1483	II
				1 $GEN^{S}CIP^{R}CLI^{S}ERY^{S}$	1 t008	ST8	Un-typable
Staff	12	12	4 (33)	4 $GEN^{S}CIP^{R}CLI^{S}ERY^{S}$	4 t008	ST8	Un-typable
Facility F							
Residents	125	125	13 (10)	13 $GEN^{S}CIP^{R}CLI^{R}ERY^{R}$	14 t002	ST5	II
Staff	80	80	5 (6)	3 $GEN^{S}CIP^{R}CLI^{R}ERY^{R}$	3 t002	ST5	II
				1 $GEN^{S}CIP^{S}CLI^{S}ERY^{S}$	1 t008	ST8	V
				1 $GEN^{S}CIP^{R}CLI^{R}ERY^{R}$	1 t2164	–	II
Total							
Residents	192	191	27 (14%)				
Staff	135	132	14 (11%)				

GEN, gentamicin; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin.

Antifungal resistance and molecular mycology

O344 Azole-resistant environmental aspergilli and *Aspergillus terreus* in Denmark, Austria and Spain

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Objectives: To investigate if azole resistant aspergilli and *A. terreus* are present in the environment and in commercial compost in Denmark (DK), Austria (A) and Spain (ES).
Methods: Soil samples were collected from the surroundings of the main hospitals in Copenhagen (CPH) (27), Innsbruck (25) and Madrid (31), flowerbeds in an amusement park in the centre of Copenhagen (23) and finally from compost bags purchased in DK (26), A (25) and ES (28).

Two grams of soil/compost were suspended in 5 ml 0.2 M NaCl-1% Tween and 100 µl plated on Sabouraud agar and 50 µl on each of 4 RPMI-1640–2% glucose agars containing itraconazole (4 mg/l), voriconazole (1 mg/l), posaconazole (0.5 mg/l) and no antifungal, respectively, and incubated at 37ºC. Identification of aspergilli was based on standard micro- and macro-morphological criteria. *Aspergillus* isolates which grew on the azole containing agars underwent susceptibility testing using EUCAST E.DEF 9.1 microdilution method for itraconazole, posaconazole and voriconazole and β-tubulin sequencing unless they were able to grow at 48ºC. The promoter and entire coding sequence of the cyp51A gene were sequenced for azole resistant isolates.

Results: From a total of 185 samples *A. fumigatus* was recovered in 139 (DK: 50/76, 66%; A: 49/50, 98%; ES: 40/59, 68%), *A. niger* in 36 (DK: 4/76, 5%; A: 5/50, 10%; ES: 27/59, 46%), *A. terreus* in seven (DK: 0/76; A: 7/50, 14%; ES: 0/59), *A. nidulans* in four (DK: 1/76, 1%; A: 0/50; ES: 3/59, 5%), *A. flavus* in three (DK: 2/76, 3%; A: 0/50; ES: 1/59, 2%), *A. lentulus* (ES: 1) and A. spp (ES: 2, molecular identification pending). A total of four *A. fumigatus* isolates (three from the amusement park in CPH and one from surroundings of CPH University Hospital (CPHUH)) displayed elevated MICs to itraconazole (>4 mg/l), posaconazole (0.5–4 mg/l) and voriconazole (4– >4 mg/l). All harboured the TR-L98H resistance mechanism. Additionally, one *A. nidulans* and one *A. niger* with elevated itraconazole MICs of 4 mg/l and 2 mg/l, respectively, were recovered from CPHUH.

Conclusion: Multi-azole resistant *A. fumigatus* is present in the environment in DK. The resistance mechanism is identical to that of environmental isolates in the Netherlands. In ES and A only *Aspergillus* species with intrinsic resistance to either azoles or amphotericin B were found. No link to commercial compost could be detected. Resistant aspergilli should be considered in aspergillosis, even in antifungal drug-naïve patients.

O345 Impact of CYP51A mutations associated with azole-resistance on *in vitro* growth rates and *in vivo* virulence of clinical *Aspergillus fumigatus* isolates

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Objectives: The emergence of multi-drug resistant *Aspergillus fumigatus* (AF) causing invasive aspergillosis has raised the question of whether the evolution of azole-resistance has an impact on virulence and therefore on clinical outcome. The aim of this study was to determine whether acquired azole resistance in fungi through mutations in CYP51A or other unknown genes comes with a cost in fitness.

Methods: The virulence study included a) 8 multi-azole resistant AF clinical isolates with different mutations in the CYP51A gene (3 strains with TR/L98H, 1 each with M220I, M220V, M220K, G54W, G138C) b) 3 wild-type azole susceptible (WT) strains as reference and c) 2 isogenic isolates with no CYP51A mutations (1 susceptible and 1 resistant strain) which were serially recovered from a single patient pre- and post-antifungal treatment. Microsatellite-typing method and CSP-method were performed to verify the genetic relationship of the 2 isolates. Susceptibility testing was performed based on the CLSI-M38A method. RT-PCR was performed to determine the CYP51A expression. *In vitro* growth rates were determined using a previously described kinetic system (Meletiadis et al JCM 2001) and defined as the incubation time needed to reach a hyphal growth of 70 microm. *In vivo* virulence was determined based on the 15-day mortality in each of 52 groups of 572 outbred i.v infected CD-1 mice (11 mice/group × 13 AF strains × 4 different CFU inocula).

Results: There was a marked reduction of virulence of the post-treatment azole-resistant isogenic isolate 2 (0% 15-day mortality) compared to a) the reference WT groups (57.6% average mortality, $p < 0.05$) b) the CYP51A groups (47.72% average mortality, $p < 0.05$) and c) the pre-treatment azole susceptible isolate 1 (90.91% mortality, $p < 0.05$). There was no difference ($p < 0.05$) in virulence for all CYP51A mutants compared to the WTs. The *in vitro* growth rates of the AF CYP51A mutants were not different compared to those of the AF WT control but it was significantly reduced when compared to the resistant isolate 2. Linear correlation was found between *in vitro* growth rates-and *in vivo* virulence (R^2 0.99, slope 2.28, $p < 0.001$). Moreover, Cyp51A elevated RNA levels implicate α-demethylase in an important role in azole resistance but not in virulence.

Discussion: Acquired antifungal resistance can lead to dramatic decrease of virulence of AF. However, CYP51A mutations associated with acquired azole resistance did not affect AF virulence.

O346 Infections due to *Candida* spp. with reduced susceptibility to caspofungin in France

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Objectives: Resistance to caspofungin in *Candida* spp. is rare with less than 25 cases reported so far in the literature. We thus analyzed the characteristics of infections due to isolates with reduced susceptibility to caspofungin collected at the French Natl Reference Center (NRCMA) between 2004 and 2009.

Methods: All *Candida* spp. received at the NRCMA are routinely tested for their susceptibility to caspofungin in AM3 medium using the EUCAST method. Those with caspofungin MIC >0.5 µg/ml (Desnos-Ollivier AAC 2008, 52:3092), were analyzed by sequencing the hot-spot regions of the FKS genes. Initial isolates were also analyzed when available and serial isolates were genotyped using polymorphic microsatellite markers. All medical charts of patients harbouring such isolates were reviewed.

Results: For the 14 episodes involving isolates with reduced susceptibility to caspofungin, patients (sex ratio M:F = 10:4, mean age: 44y) were diagnosed with candidemia (n = 8), other invasive infections (n = 4) and oropharyngeal candidiasis (n = 2). Underlying diseases were hematological malignancies (n = 6), solid organ transplant (n = 2), HIV infection (n = 1), and other conditions (n = 5). *Candida* species were 7 *C. albicans*, 6 *C. glabrata,* and 1 *C. krusei.* All 14 isolates have been recovered after the patient was treated with caspofungin for a duration ranging from 10 days to several months. All had mutation in FKS genes. Among 10 patients for whom a previous isolate was available, paired isolates shared identical genotypes. Furthermore, for each pair, the isolate recovered before caspofungin treatment had higher susceptibility (MIC < 0.25 µg/ml) to caspofungin than the second ones, and had wild-type FKS genes.

Conclusions: Caspofungin resistance in *Candida* spp. was always associated with prior exposure to the drug in severely ill patients. Further analysis should uncover if other factors such as treatment's duration influence its occurrence. Invasive infections due to various species of *Candida* spp. with acquired reduced susceptibility to caspofungin deserve attention.

O347 Innovative multiplex diagnostics for *Candida* spp. using MLPA

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Objective: *Candida* yeasts are the fourth most common source of nosocomial bloodstream infections. Especially during the last decade, the prevalence of opportunistic *Candida* infections has increased and has become a serious threat to high risk patients.

To date, most molecular diagnostic tools are based on ribosomal regions. Although broadly applied, these target genes have difficulties discriminating closely related species, causing misidentification and underestimation of polymicrobial infections. This results in an urgent need of high discriminatory and accurate detection methods for clinical purposes using novel probes.

The aim of this project is to improve molecular diagnostics of yeast-related infections.

Methods: A novel DNA based diagnostic system using Multiplex Ligation-dependent Probe Amplification (MLPA) technology serves as

our platform for molecular diagnostics using newly developed diagnostic probes.
A *Candida*-MLPA assay containing probes for *C. albicans*, *C. dubliniensis, C. krusei, C. tropicalis, C. parapsilosis, C. glabrata, C. guilliermondii,* and *C. lusitaniae* has been developed.
Results: The specificity of the assay was validated on 50 *Candida*/non-*Candida* spp. from CBS Fungal Biodiversity Centre. In addition, 95 clinical isolates were screened with the MLPA assay and the results were compared with AFLP profiles. In case of any discordance the identity of the strain was established by sequencing the ITS region. Furthermore, the total screening encompassed phylogenetically related species, including 31 *Candida* spp., 4 Pichia spp. and *S. cerevisiae.* The *Candida*-MLPA assay showed a 100% concordance with the ITS sequences. The analytical sensitivity of the *Candida*-MLPA assay was established on 100 fg gDNA, which represents less than two yeast cells.
Conclusions: We demonstrated that an assay based upon the MLPA technology was able to identify 8 *Candida* spp. in one reaction. Multiple infections, up to 7 species in one sample, could be identified. Due to the nature of the MLPA technology highly complex assays are possible providing room for additional probes like a generic *Candida* probe or probes for other emerging species.
The specificity and sensitivity of this multiparameter assay shows great potential for a fast and comprehensive screening approach for clinically relevant *Candida* spp. in a diagnostic setting.

O348 Evaluation of publicly available sequence information in the NCBI nr/nt database to identify fungi isolated from clinical samples

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Objectives: Defining the specific etiology of a fungal infection is critical to selection of appropriate antifungal therapy. Morphologic identification of fungi can take days to weeks and requires an experienced mycologist. Sequence based identification of fungi could expedite identification but it has been estimated that up to 20% of fungal sequences deposited in NCBI are incorrect. A reliable database of clinically relevant fungal sequences is critical for advancing molecular diagnosis of fungal infections. The purpose of this study was to compare morphologic identification to sequence identification of fungi isolated from clinical samples.
Methods: Fungi isolated from clinical samples at Barnes-Jewish Hospital and St. Louis Children's Hospital were identified based on morphological and/or biochemical characteristics. DNA was extracted from the fungi using the BD GeneOhm kit with a 5 min lysis followed by heating at 95 C for 15 min. The ITS1–5.8s rRNA-ITS2 region from 133 isolates was amplified and PCR products were bi-directionally sequenced on an ABI 3130. Trimmed sequences were then aligned with sequences in the NCBI nr/nt database using BLAST. Filamentous fungi and yeast from 33 genera and 55 species were included in the study.
Results: For 64 of the 133 isolates sequenced, the morphological identification matched the sequence based identification. Morphologic and sequence based identification were disparate in 38 cases. For 31 of the isolates, the sequence-based identification was equivocal; that is, the morphologic identification was found in the top ten "hits" in the sequence database, but the morphologic identification was not the first choice or there were other choices that were equal probability matches. Sequence identification performed well for most yeast species, and for some filamentous fungi, such as *Aspergillus*, *Scedosporium* and *Fusarium* spp. Sequence identification was not as accurate for identification of dermatophytes, Zygomycetes, and dematiaceous moulds.
Conclusions: Use of the NCBI sequence database for identification of clinical isolates of fungi may result in incorrect identification and administration of appropriate antifungal therapy. Once our data have been compiled into a database of fungal sequences, we will conduct challenge experiments for the identification of clinical isolates.

Legionella

S349 Population genetics, phylogeny and genomics

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Legionella pneumophila is a human pathogen that was recognized only about 30 years ago. It is the causative agent of Legionnaires' disease, a severe pneumonia that is transmitted through inhalation of aerosols of contaminated water. Shortly after its discovery, the ability of *Legionella* to multiply intracellularly in fresh water protozoa was discovered. This long lasting co-evolution between the eukaryotic host and *Legionella* has led to the selection of a panoply of virulence factors, which allow to exploit important cellular processes during infection. Compelling evidence for the importance of protozoa in the evolution of this bacterium comes from analysis of complete genome sequences. A key feature of the *L. pneumophila* genomes is the presence of a high number and wide variety of eukaryotic like proteins and protein domains probably acquired through horizontal gene transfer and/or convergent evolution. In the last years several different typing methods aiming in investigating the molecular epidemiology of *L. pneumophila* have been developed. Furthermore, the access to whole genome sequences of several *L. pneumophila* strains allowed to apply large scale comparative genomics studies using DNA arrays. A higher genetic diversity among environmental isolates with respect to clinical isolates and the presence of specific clones of *L. pneumophila* overrepresented in human disease or causing legionellosis world wide, were identified. Recently we applied new generation sequencing to further investigate the evolutionary history and population structure of the worldwide distributed epidemic and endemic clone *L. pneumophila* Paris as well as the newly emerging clone Lorraine.

S350 Cell microbiology

C.R. Roy (New Haven, US)*

We are interested in the dialogue between intracellular pathogens and eukaryotic host cells. To better understand how pathogens communicate with the host we have been studying bacterial proteins that function in the manipulation of host cellular processes. This lecture will describe proteins produced by the intracellular pathogen *Legionella pneumophila* that are delivered into eukaryotic cells by a specialized "type IV" secretion system. These bacterial proteins function to prevent fusion of lysosomes with vacuoles containing *L. pneumophila* and promote fusion of the pathogen-occupied vacuole with endoplasmic reticulum-derived vesicles. Several of these bacterial proteins modulate the activity of small GTP-binding proteins belonging to the ARF and Rab families. The biochemical activities of these bacterial effector proteins and their specificity for host targets will be discussed.

Travel medicine: an emerging field of infectious diseases

K352 Travel medicine: an emerging field of infectious diseases

P. Parola, P. Gautret, P. Schlagenhauf (Marseille, FR; Zurich, CH)*

Some 80 million individuals from industrialized nations travel to the developing world each year and it is estimated that more than 200 million people now reside outside their country of birth. In recent years, growth in international travel has been estimated at approximately 6% per year, and European travellers represent the vast majority of international travellers. Travel medicine focus on protecting the health of the individual and protecting the wider community in which that individual lives, works or travels. Initially derived from infectious diseases and tropical medicine, the specialty now encompasses primary care, migrant medicine, occupational medicine, wilderness medicine, as well as international health.

Travelers can spread new and reemerging infectious diseases that initially appear in developing countries, and act as ideal sentinels for the early detection of these diseases. Over the past decade, the global public health community has been facing the challenges brought on by the emergence and rapid worldwide spread of novel influenza strains, SARS, chikungunya virus, and drug-resistant tuberculosis amongst other pathogens. Modern transportation and the growth of tourism, immigration, and business travel were factors that contributed to dissemination of these high impact pathogens.
Specialized travel/tropical medicine clinics in Europe are ideally situated to effectively detect emerging infections and to track ongoing trends in travel-related illness and emerging agents in collaboration with laboratories of microbiology. Over the past decade, both global and regional provider-based surveillance networks have emerged that have provided, for the first time, systematic and robust data that define the spectrum of illness and the places of exposure to the most significant health risks that face travelers.

Infection control: zero tolerance?

S354 Zero tolerance: an impossible task

W. Graninger (Vienna, AT)*

With a declining arsenal of antibiotics to treat infections, it was increasingly clear that the traditional orientation toward control of HAIs (hospital acquired infections) needed to shift to one where preventing the occurrence was the priority throughout the institution.
There has to be a global capacity for the political, financial, managerial, and technical support needed for worldwide initiatives. These considerations were all addressed when diseases like smallpox, polio, measles, dracunculiasis, lymphatic filariasis, onchocerciasis, Chagas disease, and Hansen disease were targeted for eradication. This model doesn't exactly work for HAIs, because we deal with many different organisms and types of infections, humans are not the only reservoir, many of the organisms are normal flora, and relatively limited scientific and operational resources have actually been dedicated to preventing HAIs.
New technologies and procedures, more virulent pathogens and increasing resistance will continue to challenge the healthcare community in its efforts reduce HAIs.
If one looks theoretically at all of the infections that occur, one could divide them up into two main groups; some that are preventable and some that are not. The ones that are preventable are preventable through the implementation of practices, behaviors and procedures, and following a very strict clinical pathway. Others are not probably preventable, and that is the irreducible amount.
The 'getting to zero' movement is the product of three forces: the expansion of external pressures on infection control programs, the intrusion of suboptimal evidence, and the convergence of quality improvement and infection control. The 'zero' obsession has a number of worrisome, unintended consequences. It sets up unrealistic expectations on the part of the public and healthcare administrators, leading to unreasonable demands on infection control programs. It fosters a punitive culture, since someone must be at fault for causing infections. It separates infection control from safety and quality, when infection control concerns trump other important safety issues. It has shifted the development of interventions away from an approach based on local risk assessment to the promotion of a one-size-fits-all approach. Healthcare workers and hospital epidemiologists have become demoralized when the expectations for getting to zero persist but the elusive zero has not been attained.
The concept of "targeting zero HAIs" is controversial, because many people believe it sets unrealistic or impossible expectations that all HAIs are preventable and that any HAI that may occur was due to an error or a broken process. Targeting 'zero' is problematic, because it does not address the variation in the risk of HAIs in different patient populations or settings, it does not address the denominator or time frame that is necessary to understand rates of infection, and it inherently seems scientifically unrealistic.
"Getting to zero" is a sound bite that misleads the public and is not helpful to hospital epidemiologists. The "zero" approach to HAIs is rigid, dishonest, and anti-intellectual, and it dives a culture of blame.

MRSA screening: what else to know

S355 Light and shadow of new tests

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Healthcare associated staphylococcal infection represents a major health and economic burden world wide. The *in vitro* diagnostic industry has in recent years invested significantly to develop and market advanced SA/MRSA screening tests for clinical and public health decision support in healthcare facilities. Successful technology innovation meets longstanding medical needs of timely detection of SA/MRSA carriers to accelerate control of transmission. The purpose of SA/MRSA screening is two-fold: (1) to adapt individual therapeutic and prophylactic treatment regimens and (2) to isolate and decolonise carriers early on to reduce secondary nosocomial transmission. Novel screening tests include enhanced culture-based methods and molecular-based DNA detection methods. Significant enhancement of culture based MRSA screening has been achieved with marketing of new generation of selective, cefoxitin-containing chromogenic agar media. The best performing media provide MRSA detection within 16 to 48 h post sampling with variable sensitivity (ranging 50–99%) and good to excellent specificity (>95%). A more sophisticated, semi-automated system of luminescent growth detection in selective liquid media proved insufficiently sensitive in clinical trials. Several PCR systems are commercially available for MRSA screening. PCR assay time varies from 75 min to 6 h. Analytical complexity ranges from multi-step manual procedure of sample preparation, DNA extraction, amplification and hybridisation to fully automated systems with minimal sample preparation. Diagnostic accuracy of PCR tests varies according to test, test version, sample site and reference method used as gold standard with sensitivity range of 70–100% and specificity range of 90–99%. Impact of rapid MRSA screening on transmission control effectiveness depends on many contextual factors, including workflow integration and clinical turn-around time, case-mix and type of care, MRSA incidence and prevalence, infection control policy, compliance with infection control measures and intervention study design. Cost-effectiveness evaluation should carefully examine these parameters for each test before routine implementation.

The threat of a new influenza A pandemic

S358 A "One Health" approach to influenza virus infections to support public health

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The emergence and spread of the 2009 pandemic H1N1 virus (H1N1 2009) from the animal reservoir raises questions on the future approach to influenzavirus infections. We have evidence demonstrating that influenzavirus genes migrate across continents and animal species, and assemble themselves in combinations which are a threat to animal and human health, resulting in panzootics like H5N1 or pandemics like H1N1 2009. The latter contains a unique combination of genes from three species and two hemispheres. In a globalized environment, mapping gene movement across species and national borders and identifying mutations and gene constellations with pandemic potential or virulence determinants is essential to enact prevention and control strategies at a global level. This is in line with, and possibly the best example of, the 'One Health' vision: a multidisciplinary collaborative approach to improve the health of humans, animals and the environment endorsed by the UN Food & Agriculture Organisation, the World Organisation for Animal Health (OIE) and the World Health Organisation.
Vast improvements in capacity building have been achieved as a result of the H5N1 global crisis. Thousands of viral isolates with zoonotic

potential have been obtained through surveillance efforts, although the genetic information has not been exploited fully. In addition, the circulation of influenzaviruses in certain species including dogs, pigs and horses has been neglected.
Time has come to invest in a novel approach to influenzavirus infections, abandoning prefixed compartments linked to geographical origin or species of isolation, and analyse the influenza gene pool as one entity. We propose capitalising on existing achievements and investments to develop an international network and a permanent observatory which will improve our understanding of the dynamics of the influenzavirus gene pool in animals and humans. This will generate essential information to support both public and animal health.
The "One Flu" initiative would result in international synergies, bridging gaps between medical and veterinary scientists, permanent monitoring of virus evolution and epidemiology and the best exploitation of investments in capacity building. Above all it could be a challenge and opportunity to implement the "One Health" vision, and possibly act as a model for other emerging zoonotic diseases.

Antimicrobial activity against carpabenem resistant Gram-negative bacteria

O359 *In vitro* activity of antimicrobials in combination against clinical strains of extreme drug-resistant *Acinetobacter baumannii* to all antibiotics including polymyxin B in Singapore

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Objectives: We have used polymyxins since 1990s in Singapore. Emergence of extreme-drug resistant(XDR) *Acinetobacter baumannii*(AB) infection resistant to all available antibiotics including polymyxins have finally occurred. Combination therapy may be the only viable option until new antibiotics become available. We assess the *in vitro* activity of various antimicrobials and elucidate the most effective combination therapy against these XDR AB.
Methods: Five XDR AB strains from 4 different patients (1 with haematological malignancy and 3 with severe burns injuires) were identified, after weeks of polymyxins therapy. MICs were determined according to a modified CLSI broth-dilution method. Time-kill studies (TKS) were performed with approximately 10^5 CFU/ml at baseline with the maximum, clinically achievable, unbound concentration (mg/L) of PB (2), (R)rifampicin (2), (M)meropenem (64), (C)cefepime (200) and (T)tigecycline (2) alone and in combination against these 5 isolates.
Results: All isolates were resistant to all antibiotics including PB (MICs 16–128 mg/L). In TKS, CP provided a killing effect of >99% (>2 log kill) from baseline inoculum at 24 h for all 5 strains. CM & MR exhibited killing effect of >99% from baseline inoculum at 24 h for 4 strains. MP exhibited killing effect of >99% from baseline inoculum at 24 h for 3 strains. CR, TM & PR exhibited killing effect of >99% from baseline inoculum at 24 h for 2, 1, & 1 strains, respectively. Four strains had 3 or more antibiotic combinations that provided killing effect of >99% from baseline inoculum at 24 h, while 1 strain had only 1 antibiotic combination that was effective.
Conclusions: Clinical isolates of AB resistant to PB is also resistant to all major antibiotic classes with no compromise in biofitness; in contrary to previous reports that illustrate PB resistant AB with a substantial deficit in biofitness *in vitro*. We had shown that CP, CM, MR, MP may be potential antibiotic combinations as pre-emptive therapy for XDR AB infections and the effective combinations were strain specific. Our study warrants further investigations.

O360 *In vitro* antibacterial activity of ceftazidime in combination with the β-lactamase inhibitor NXL104

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Objective: NXL104 is a novel β-lactamase inhibitor that has been shown *in vitro* and *in vivo* to inhibit both class A and class C β-lactamases; it is currently in phase 2 of clinical development in combination with ceftazidime (CAZ). The objective of the study was to evaluate the antibacterial activity of CAZ/NXL104 combination against characterized Enterobacteriaceae species.
Methods: Activity of CAZ/NXL104 combination was tested on 222 strains of Enterobacteriaceae representing most clinical species. The panel included 49.5% CAZ-resistant isolates, most with characterized β-lactamases. Minimal Inhibitory Concentrations (MIC) were determined with NXL104 at fixed ratios of 1:1 to 16:1, or at fixed concentrations of 2, 4, and 8 μg/mL.
Results: CAZ/NXL104 combinations were active against all isolates and had significantly lower MICs than CAZ alone, cefotaxime, ceftriaxone, cefepime, or piperacillin/tazobactam (MIC90s >128 μg/mL). MIC50s and MIC90s for CAZ with fixed concentrations of NXL104 were ⩽0.125–0.5 and 1–4 μg/mL, respectively, and 1–2 and 4–16 μg/mL, respectively, with fixed ratios. Potentiation of CAZ by NXL104 was generally 16 to 512-fold for TEM, SHV, CTX-M or KPC producers. Against KPC producers, NXL104 reduced CAZ MICs from >128 to ⩽0.25–8 μg/mL. Potentiation of 8 to 512-fold was observed against AmpC enzyme producers.
Conclusion: The CAZ/NXL104 combination exhibits a broad spectrum activity against resistant Enterobacteriaceae isolates, and represents an important next-generation of β-lactam/β-lactamase inhibitor combination.

O361 Activities of tigecycline in combination with colistin or meropenem against KPC carbapenemase-producing Enterobacteriaceae by time-kill analysis

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Objectives: Enterobacteriaceae producing KPC carbapenemases cause hospital infections often associated with therapeutic failures and increased mortality. The treatment of these infections usually requires the use of tigecycline or colistin as a last-resort drug. The objective of this study was to test the *in vitro* activity of tigecycline against KPC-producing Enterobacteriaceae.
Methods: The KPC-producing isolates comprised four *Klebsiella pneumoniae*, two *Escherichia coli*, one *Enterobacter cloacae* and one *Serratia marcescens*. The *K. pneumoniae* and *E. coli* isolates were randomly selected among those representing different clonal types. MICs were obtained using the macrodilution method in fresh Mueller–Hinton broth (MHB), before performing time-kill assays by inoculating 5x105 CFU/mL of the test organisms in 3 ml MHB. *E. coli* ATCC 25922 was used as control. Antibiotics (tigecycline, colistin and meropenem as single agents and in combinations) were added at concentrations 1x and 2x the MIC for each isolate. Aliquots were removed at times 0, 2 h, 4 h, 6 h, 8 h, 16 h and 24 h post-inoculation, serially diluted and plated on MH agar plates for enumeration of viable colonies. Each time-kill experiment was performed twice. As bactericidal activity was defined a ⩾3 log10 CFU/mL reduction in viable cells with respect to the original inoculum.
Results: Macrodilution MIC values were 0.25 to 2 mg/L for tigecycline, 0.5 to 1 mg/L for colistin and 2 to 16 mg/L for meropenem. In time-kill assays, tigecycline and meropenem as single agents were mostly bacteriostatic for the first 6–8 hours of incubation with bacterial regrowth to follow, while colistin alone was ineffective. The tigecycline plus colistin combination was in most cases bactericidal after 4 to 8 h of incubation and in some cases also synergistic compared with

tigecycline alone, although bacterial regrowth was observed after 8 to 16 h. The tigecycline plus meropenem combination was in most cases also bactericidal after 4 to 6 h of incubation and thereafter regrowth was observed.

Conclusion: The results of the present study indicate that tigecycline alone could be a therapeutic option for infections due to multidrug resistant KPC producers when bacteriostatic activity is adequate or in combination with colistin or meropenem when bactericidal activity is necessary. Additional *in vivo* tests may be warranted to fully assess the killing kinetics of tigecycline against KPC producers when the immune system is competent.

O362 Activity of temocillin against carbapenem-resistant clinical Enterobacteriaceae

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Objectives: Until recently, carbapenems retained near-universal activity against Enterobacteriaceae but resistance is now emerging, mediated either by combinations of ESBL or AmpC enzyme and porin loss or by carbapenemases. The prevalent emerging carbapenemases vary by country: KPC enzymes in the USA and Israel, OXA-48 in Turkey, VIM in southern Europe and NDM in Italy. The UK sees small numbers of isolates with each of these types, some imported, some representing domestic spread. Most are multiresistant. We examined their susceptibility to temocillin a 6-α-methoxy derivative of ticarcillin, notable for stability to ESBLs and AmpC.

Methods: The 81 isolates tested isolates variously had KPC (n = 10), SME-1 (1), IMP (13), VIM (5), NDM (17) or OXA-48 (19 carbapenemases or had combinations of impermeability with an AmpC enzyme or ESBL (16); they included 52 *Klebsiella* spp., 18 *Enterobacter* spp., 6 *E. coli* and 5 others. Transformants and transconjugants were prepared in *E. coli* DH5-α and J62–1, respectively. Carbapenemase genes were identified by PCR and sequencing; MICs were measured by CLSI agar dilution.

Results: MICs for isolates with KPC carbapenemases were from 16–64 mg/L (geom. mean 40 mg/L) and introduction of a KPC+ plasmid into *E. coli* only raised the MIC from 8 to 16 mg/L; by contrast MICs for isolates with metallo-β-lactamases were >128 mg/L in 22/36 cases, and introduction of plasmids coding NDN carbapenemase (the commonest MBL in the UK) into *E. coli* DH5α raised the temocillin MIC from 4 to >64 mg/L. Temocillin MICs for isolates with OXA-48 enzyme were >128 mg/L in 18/19 cases, and the MIC shift on transformation of *E. coli* DH5-α was from 4 to >128 mg/L. Temocillin MICs for isolates with combinations of porin loss and AmpC or an ESBL were from 8 to 128 mg/L (geom. mean 25 mg/L).

Conclusion: If the dosage can be raised from the present 2 g bds, temocillin may be a therapeutic option in some infections due to Enterobacteriaceae with KPC carbapenemases or combination of AmpC or ESBL and porin loss, not against those with OXA-48 or metallo-carbapenemases.

O363 Interactions mediating the positioning of mercapto-phosphonate inhibitors in the active site of metallo-β-lactamases

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Objective: Metallo-β-lactamases (MBL) represent an emerging problem due to their capacity to hydrolyze almost all β-lactam antibiotics, including last generation cephalosporins and carbapenems. Due to the presence of two chelating functions (sulfanyl and phosphonato), the mercaptophosphonic acids (phosphorous analogues of mercaptocarboxylic acids) are potential candidates for MBL inhibitors.

Methods: The inhibitory effect of 14 mercapto-phosphonate derivatives against representatives of the three subclasses of MBLs (VIM-4 (B1), CphA (B2) and L1 (B3)) was previously reported [1]. Here, in order to determine the interactions mediating the positioning of the inhibitors in the active site of each enzyme, crystallographic and docking studies were performed with 10a and 18, both inhibitors being active against the three subclasses.

Results: The crystallographic structure of the CphA-10a and CphA-18 indicated that the sulphur atom of 10a and the phosphonato group of 18 interact with the zinc ion respectively. Molecular modelling on the VIM-4 (B1) and FEZ-1 (B3) enzymes with 10a and 18 also brought to light different binding modes depending on the enzyme and the inhibitor, consistent with the crystallographic structures.

Conclusions: The investigation of mercapto-phosphonate derivatives as MBL inhibitor has allowed us to find potent inhibitors active on representative members of all the three MBL subclasses. Moreover, on the basis of structural and modelling data, the inhibitory strength of these compounds will be improved further.

Reference(s)

[1] Lassaux P., Hamel M., Gulea M., Mercuri P., Horsfall L., Bebrone C., Gaumont A-C., Frère J., Galleni M. Mercapto-phosphonate compounds as broad-spectrum inhibitors of the metallo-β-lactamases, Abstract number: 1732_295, ECSMID 2007.

Infection in the immunocompromised host

O364 *Hormographiella aspergillata*: an emerging mould in acute leukaemia patients?

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Objectives: Invasive fungal infections (IFI) are a major cause of morbidity and mortality in hematological patients. The emergence of non-*Aspergillus* molds has been observed in recent years. *Hormographiella aspergillata* is a Basidiomycete and has previously been implicated in human infections rarely. We describe 3 cases of IFI with *H. aspergillata* occurring within one year on the hematological ward at the University Hospital Basel.

Methods: Patients were hospitalized in single rooms with special high-efficiency particle air filters. Surveillance fungal cultures of air filters were carried out regularly. No primary mold prophylaxis was performed. Screening for mold infections was performed by twice weekly measuring serum galactomannan and weekly pulmonary CT scans. Sabouraud agar was inoculated for fungal cultures. The identification of *H. aspergillata* by culture was molecularly confirmed. Susceptibility testing was performed by Etest.

Table 1. Minimal inhibitory concentrations of antifungals against *Hormographiella aspergillata* patients isolates

Compound	Case 1	Case 2	Case 3
Amphotericin B	not done	0.5 mg/l	0.5 mg/l
Caspofungin	not done	2 mg/l	>32 mg/l
Voriconazole	not done	0.125 mg/l	0.25 mg/l
Fluconazole	not done	not done	>256 mg/l
Posaconazole	not done	not done	not interpretable

Results: In one year, 3 cases of pulmonary IFI with *H. aspergillata* in leukemic patients, undergoing prolonged periods of severe neutropenia, were found. One patient (case 1) also had cerebral and ocular involvement in autopsy. This patient had positive blood cultures with *H. aspergillata.* In all patients pulmonary CT showed infiltrates, bronchoalveolar lavage performed failed however to identify the causative microorganism and serial serum galactomannan measurements remained negative. In contrast, specimens collected by VATS grew *H. aspergillata* in both patients where it was performed (cases 2+3). *In vitro* susceptibility testing was done in 2 cases (Table 1). Treatment consisted of caspofungin in one patient (case 1), after severe hepatotoxicity due to azoles, and of second generation azoles in the others. An evaluation of the *in vivo* efficacy of antifungal treatment in this series is difficult. All patients died, one due to the disseminated

IFI (treated with caspofungin), 2 due to progression of the underlying hematological disease and allogeneic transplant related complications.
Conclusions: Despite the accumulation of 3 cases of an unusual IFI with *H. aspergillata,* no hospital source was detected. Due to the rarity of cases reported there is no established treatment for *H. aspergillata.* Standardized antifungal susceptibility testing of filamentous fungi has been established only recently and breakpoints with proven clinical relevance have yet to be identified. Voriconazole might be a valuable treatment option.

O365 How to improve microbial documentation in febrile neutropenia? Impact of implementing an automat in the ward and addition of DNaemia detection

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Objectives: Febrile neutropenia (FN) is the most frequent complication of high-dose chemotherapy-induced neutropenia. Only 30% of FN episodes are microbiologically documented by routine procedures, mainly by blood cultures (BC). We wondered whether shortening the time before incubation could improve this figure. In addition, we searched microbial DNA by the Septifast® test.
Methods: Adult patients were eligible at their 1st FN episode if they were neutropenic (PMN $< 0.5 \times 10^9$/L), febrile ($\geqslant$38º3 or twice $\geqslant$38º within 8 h), and had a central venous catheter (CVC). At onset of fever, we collected 4 BC bottles (2 aerobic and 2 anaerobic)sampled through the CVC. They were immediately incubated in the ward (BacT/Alert3D®). Septifast was performed on 1.5 ml of blood sampled at the same time. In parallel, 2 standard BCs (1 aerobic and 1 anaerobic bottles) were routinely sampled (1 CVC and 1 peripheral) and sent to the laboratory for incubation. Each patient was assessed for microbial documentation in the blood, either BC positivity or DNA detection; and the time elapsed between blood sampling and documentation, when this latter was positive. Pairwise analyses were performed using the McNemar test for documentation rate and the Wilcoxon signed-rank test for times for positivity.
Results: 120 consecutive FN episodes were included from Feb 2008 to Mar 2009: 97 (80%) acute leukemia/myelodysplasia, 26 (17%) lymphoid malignancies and 4 (3%) others with 18 (15%) episodes in allogeneic and 16 (13%) in autologous stem cell transplantation. The rate of BC positivity was 30% (36 episodes) in the study process and 28.3% (34 episodes) in the routine process (McNemar's $\chi^2 = 0.67$, $p = 0.41$). In the microbiologically documented episodes, the time elapsed between incubation and positivity was significantly shorter for the BC bottles incubated in the ward (median rank: 12 h25 min [7 h55–25 h37]) than for the routine BC bottles (median rank: 13 h03 min [9 h31–43 h33]) ($p = 0.002$). The Septifast test was positive in 9 episodes (7.5%) that were positive also by the BC testing. In 8 additional episodes, the Septifast® test showed low DNA content out of the threshold, which was nevertheless concordant with positives BCs.
Conclusion: Immediate incubation of blood cultures in the ward and detection of DNaemia with the Septifast test did not improve the rate of microbial documentation for FN episodes when compared to BC routine process. However, it reduced the time to positivity.

O366 Aetiology of eye infections in hospitalized ophthalmological patients

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Objectives: Ophthalmological patients are prone to nosocomial infections due to advanced age and common underlying medical conditions. Eye infections caused by multi-drug resistant (MDR) pathogens may further contribute to loss of vision in already visually disabled persons. Antimicrobial therapy is often difficult due to unsatisfactory penetration of many agents into the eye. Empiric therapy should be based on data comprising most prevalent pathogens, including MDR pathogens.
Methods: Specimens for bacteriological and fungal culture were obtained from patients hospitalized in ophthalmological hospital (2005–2009). Samples were cultured and isolates identified according to standard microbiological techniques.
Results: In total 1662 specimens were cultured, including swabs from conjunctiva, cornea, throat, nose, wounds and cornea conservation medium. The samples yielded 328 isolates, comprising 293 strains of bacteria and 7 strains of yeast-like fungi. Among bacteria predominated staphylococci – 197/293 (67.23%), followed by enteric rods – 36/293 (12.29%), non-fermenting rods – 20/293 (6.87%) and streptococci – 19 (6.48%). Among alarm pathogens there were methicillin-resistant *S. aureus* (MRSA) strains (4/50, 8.00%), high-level aminoglycoside resistant (HLAR) strains of enterococci (1/8, 12.50%), extended-spectrum β-lactamase producing – ESBL(+) enteric rods (2/36, 5.56%) and ESBL(+) Gram-negative nonfermenting rods (4/20, 20.00%). Methicillin-resistant coagulase-negative staphylococci (MRCNS) comprised 28/147 (19.05%) strains. Among all cultured microbial isolates there were 7/300 (2.33%) strains of fungi. Predominated *Candida parapsilosis* (4/7 strains), followed by *C. albicans* (2/7) and *C. glabrata* (1/7).
Conclusion: In ophthalmological specimens predominated Gram-positive cocci, mainly staphylococci. The presence of MRCNS (19%) may be important in therapy of biofilm-associated implant infections. In this study there was a high percentage (20%) of ESBL(+) Gram-negative nonfermenting rods. Among yeast-like fungi *Candida parapsilosis* was more often isolated than *C. albicans*. This is clinically relevant as *C. parapsilosis* tends to form a biofilm on artificial materials, increasingly used in ophthalmic surgery.

O367 Prognosis of human listeriosis is directly associated with the severity of the underlying disease

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Objectives: To determine the incidence, manifestations, outcome and risk factors for mortality of a series of adult patients with listeriosis.
Methods: Retrospective review of medical records of adult patients with isolation of *Listeria monocytogenes* from blood, CSF or other sterile fluid during a period of 15 years in two hospitals in Madrid.
Results: Sixty four patients were assessed. The mean age was 58.8 years (from 20 to 85) without differences in distribution by genders. The prevalence of infection was 0.5 cases per 100.000 inhabitants year. Seventy per cent of patients had a chronic underlying disease: cirrhosis of the liver (30%), hematologic neoplasms (25%), chronic debilitating conditions (23%), collagen vascular disease (13%) and solid neoplasms (11%) were the most common underlying conditions. Only 1 pregnant woman was seen. Primary bacteremia (56%) and meningoencephalitis (44%) were the most common presentations. In addition, 10 patients developed focal infección such as bacterial peritonitis (4), endocarditis (3) and brain abscesses (3). Although most infections were community-acquired, 17 episodes (26%) developed within the hospital after more than 72 h of admission. Nosocomial outbreaks were not detected. Ampicillin (34%) alone or in combination with gentamicin (44%) and cotrimoxazol (16%) were the antimicrobial regimens most frequently used. We did not find differences in the mortality rate among patients on monotherapy (32%) and combined therapy (35%). Cotrimoxazol was successfully used in 9 out of 10 patients treated. Nineteen patients (39%) died within 30-days of diagnosis. Mortality was significantly higher in patients with hematologic or solid neoplasms than in those with cirrhosis or other underlying debilitating disorders (64% vs 36%, $p < 0.05$). All the patients without comorbidities survived the infection.
Conclusions: Human listeriosis is a severe disease still producing high mortality particularly in immunocompromised hosts with hematologic and solid neoplasms and other debilitating conditions. Despite synergistic *in vitro* activity, the combination of ampicillin plus gentamicin did not determined survival benefits. Cotrimoxazol may be a useful alternative for the antimicrobial therapy of human listeriosis.

O368 *Campylobacter* sp. bacteraemia in Taiwan, 1999–2008

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Objectives: Bacteremia caused by *Campylobacter* species is rarely described in Asia. We investigated the bacteriology and clinical presentation of *Campylobacter* bacteremia in Taiwan.
Methods: There were 28 episodes of *Campylobacter* spp. bacteremia in 28 patients occurred from January 1999 to December 2008 in National Taiwan University Hospital. All the 28 isolates were identified by conventional phenotypic methods and later confirmed by using previously described multiplex PCR (cadF, hipO and asp gene detection) and 16S RNA gene sequencing up to 900 base pairs. Medical records were reviewed in 24 patients.
Results: The most common species is *C. coli* (N = 17[60.7%]) followed by *C. fetus* and *C. jejuni* (N = 6[35.2%] and N = 5[29.4%]; respectively). One isolate was identified as *C. coli* by multiplex PCR which is not consistent with the result of 16S RNA gene sequencing *(C. jejuni* strain SWUN0713; accession number: gb GQ479815.1). Of the 24 cases with available medical records, 17 patients were male with a median age of 45.7 (range of age was from 7.75 to 80.67 years old). The main underlying conditions were hemato-oncological disease (41.67%) followed by hepatic decompensation (37.5%), renal insufficiency (33.3%) and liver cirrhosis(25%). There were 7 patients (29.17%) were in usage of steroid. The most common clinical manifestations were intra-abdominal infection (54.16%), primary bacteremia(10 patients, 41.67%), and cellulitis (4.1%). The mean Pitts bacteremic score was 1.58 (range from score 0 to score 9). There were 6 patients' systolic blood pressure were below 90 mmHg(25%). And consciousness change were noted in 8 patients (33.33%). Leucocytosis were noted in 12 patients(50%), and the mean value of white blood cell counts were 11,065.5/ul (Range was from 4,050/ul to 21,340/ul). And the mean value of c-reactive protein was 6.56 mg/dl. Third generation antibiotics was the most commonly used empirical treatment regimen (50%) and Amoxicillin/clavunalic acid was 20.83%. The mean hospitalization stay was 12.96 days. All cause mortality at 14 days was 4.16% (N = 1) and 16.67% (N = 4) at 30 days.
Conclusions: *Campylobacter coli* is the leading species causing invasive Campylobacteriosis in immunocompromised patients in Taiwan.

Experimental treatments in animal models

O369 Antifungal drugs improve survival by immunomodulation rather than by reduction of fungal burden in experimental cerebral aspergillosis

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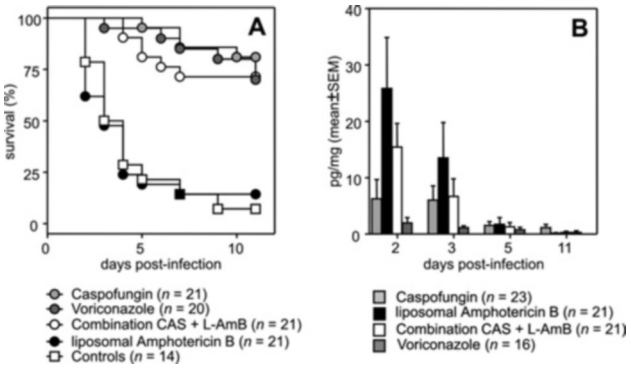
Objectives: To evaluate the antifungal and immunomodulatory effects of antifungal drugs we used a lethal model of cerebral aspergillosis in non-immunosuppressed infant rats.
Methods: Eleven-day-old male Wistar rats were infected by intracisternal injection of 7.17 log10 colony-forming units of *Aspergillus fumigatus* conidia. Treatment started 22 h after infection and was given for 10d. Regimens were (i) caspofungin (CAS) 1 mg/kg/d i.p. qd, (ii) liposomal amphotericin B (L-AmB) 5 mg/kg/d i.p. qd, (iii) both drugs combined at the same dose, and (iv) voriconazole (VCZ) starting at 15 mg/kg bid and increasing the dose to compensate for auto induction of metabolism. In survival experiments censored at 11d brains were examined at the time of death. To monitor cerebral disease progression, animals were sacrificed 2, 3, 5, and 11d after infection. Brain homogenates were analyzed by quantitative fungal culture, a flow-cytometry based assay for cytokine quantification, and ELISA for galactomannan (GM) detection. Animals with symptoms of severe disease were sacrificed for ethical reasons.
Results:

1. Survival experiments [Fig. A]: Compared to controls (4.4±2.7d), survival times were significantly increased by treatment with CAS alone (10.3±1.7d; $p < 0.0001$) and combined with L-AmB (9.3±2.8d; $p < 0.0001$) as well as VCZ (10.1±2.2d; $p < 0.0001$). In contrast, survival time of animals treated with L-AmB alone (4.3±3.1d) was not different from untreated controls.
2. Fungal culture: The cerebral fungal burden declined over time in all animals including untreated ones. Interestingly, there was no significant difference between controls and treated animals.
3. GM: GM peaked later than the CFU counts in all treatment groups except L-AmB. There were no significant differences in GM indices regarding treatment and time.
4. Cytokines: At 2d after infection both IFN-γ and TNF-α [Fig. B] levels were significantly higher in animals treated with L-AmB alone (135.8±93.9pg/mg and 25.8±22.2pg/mg) compared to CAS (24.1±20.8pg/mg and 6.3±8.4pg/mg; $p < 0.001$), and VCZ (13.8±8.0pg/mg and 2.0±1.9pg/mg; $p < 0.001$). No differences were found for IL-1α and IL-10. IL-6 was undetectable in most animals but elevated in severe disease.

Conclusion: In this lethal model of cerebral aspergillosis mortality is not determined by the antifungal drug's effect on cerebral fungal burden but by its modulation of the host's immune response.



O370 Receptor for advanced glycation end-products is protective during murine tuberculosis

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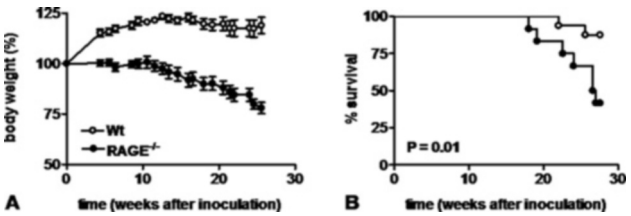
Objective: The development of active tuberculosis after infection with *Mycobacterium* (M.) tuberculosis is almost invariably caused by a persistent or transient state of relative immunodeficiency. Multidrug-resistant strains are on the rise and the frequent occurrence of co-infection with the human immunodeficiency virus makes the treatment and outcome of tuberculosis even more worrisome. The receptor for advanced glycation end products (RAGE) is a promiscuous receptor that is involved in pulmonary inflammation and infection. We here aimed to investigate the role of RAGE in tuberculosis.
Methods: RAGE deficient (RAGE–/–) and normal wild-type (Wt) mice were intranasally inoculated with live virulent *M. tuberculosis*.



Results: While lungs of infected Wt mice expressed RAGE, in particular on endothelium, *M. tuberculosis* pneumonia was associated with an enhanced expression of pulmonary RAGE. Lung inflammation was increased in RAGE–/– mice, as indicated by histopathology, percentage of inflamed area, lung weight and cytokine and chemokine levels. In addition, lung lymphocyte and neutrophil numbers were increased in the RAGE–/– mice. RAGE–/– mice displayed higher mycobacterial loads in the lungs after 3 weeks of infection, while they showed similar loads

in the liver at 3 and 6 weeks. Finally, RAGE–/– mice displayed body weight loss and a worsened *M. tuberculosis* induced mortality (Figure).
Conclusion: These data suggest that RAGE plays a beneficial role in the host response to pulmonary tuberculosis.

O371 Imipenem or meropenem plus clavulanate combination improves survival of mice infected with *M. tuberculosis*

N. Veziris, C. Truffot, J. Mainardi, V. Jarlier (Paris, FR)*

Background: Due to the emergence of extensively drug resistant strains (XDR) of *M. tuberculosis*, new antituberculous agents are desperately needed. Although β-lactam antibiotics are not considered as antituberculous drugs, it has been recently shown *in vitro* that the combination of meropenem and clavulanate has synergic activity against *M. tuberculosis*.
Objectives: We wished to evaluate in the murine model of tuberculosis the activity of penems alone and combined with clavulanate against *M. tuberculosis*.
Methods: Swiss mice were infected intravenously with 3.105 *M. tuberculosis* H37Rv and treated the day after inoculation for 4 weeks. Treatment groups consisted of 10 mice. The test groups were treated with clavulanate (100 mg/kg) alone, imipenem (100 mg/kg) and meropenem (100 mg/kg) alone or combined with clavulanate whereas a positive control group was treated with isoniazid (25 mg/kg) and a negative control group was held without treatment. At the end of treatment, surviving mice were sacrificed and lungs harvested. Treatment efficacy was assessed on survival rate, spleen weights and lung CFU counts.
Results: at the end of the 4 weeks, the mortality rates were the following: untreated 60%, isoniazid 0%, clavulanate 30%, imipenem 10%, meropenem 30%, imipenem+clavulanate 0%, meropenem+clavulanate 0%. The combination of imipenem or meropenem plus clavulanate significantly improved survival ($p = 0.01$ vs untreated mice). On the other hand, imipenem and meropenem combined with clavulanate did not prevent splenomegaly (591 and 573 mg) whereas isoniazid did (304 mg) ($p > 0.05$). Among groups of mice with 100% survival, only isoniazid reduced lung CFU counts ($-1.2 \log_{10}$ CFU vs D0), the penem+clavulanate combinations did not prevent bacterial growth ($+0.9$ to $1.4 \log_{10}$ CFU vs D0).
Conclusion: although less active than isoniazid, the combinations of imipenem or meropenem and clavulanate improve survival of mice infected with *M. tuberculosis* and should be further evaluated.

O372 Evaluation of the efficacy of anti-staphylococcal human therapies in a severe PVL pneumonia model

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Objectives: Many strains of *S. aureus* harbour the Panton Valentine Leukotoxin (PVL) phage and can cause severe necrotising pneumonia in young immunocompetent patients. *In vitro* studies showed that some anti-staphylococcal drugs can induce or inhibit the PVL production. Animal models are needed to evaluate new treatment strategies in such infections. We compared the efficacy of 4 antibiotic human therapies in a severe model of PVL necrotizing pneumonia in rabbits.
Methods: 2 isogenic and fully-susceptible *S. aureus* strains were used to induce pneumonia: RN6390 PVL– and LUG855 PVL+. Animals were randomized to no treatment (controls) or to a 48 h IV human equivalent dosage of either vancomycin (VA continuous perfusion Css=30 mg/l), cloxacillin (CLOX 2g/6 h), clindamycin (CLI 600 mg/8 h) or linezolid (LZO, 600 mg/12 h). Serum levels were measured by microbiological assay or HPLC, pharmacokinetic data were obtained, and evaluation of efficacy was based on bacterial counts in lungs (mean±SD) and the residual quantity of PVL (μg/residual CFU).
Results: Several inocula were tested to perfect this model. Here are the results for an inoculum of 8.5 log CFU/mL (table).
Controls: The mortality rate (60–66%) and bacterial content in controls were the same between PVL– and PVL+ groups.
Bacterial reduction: No significant efficacy was obtained with VA. LZO, CLOX and CLI were highly efficacious against both strains with a complete sterilization of lungs and spleen for CLOX and CLI (CLOX = CLI > LZO > VA).
Early mortality: VA did not reduce the mortality rate. 0% of early mortality was obtained with CLOX and CLI. LZO slightly reduced the mortality rate (CLOX = CLI > LZO > VA).
PVL assessment: VA and CLOX did not reduce the residual PVL quantity significantly. LZO reduced by half the PVL quantity. CLI was strongly effective on pulmonary PVL reduction (CLI > LZO > CLOX > VA).
Conclusion: No difference was noted in both control groups PVL– or PVL+ in terms of pathogenic effect (mortality, CFU, macroscopic score ...) probably due to other virulence factors. VA was slightly efficacious in this model. CLOX was strongly bactericidal contrasting with the persisting production of PVL. LZO and CLI reduced the PVL production and the residual bacterial content in this model. Other studies are under way to evaluate the impact of such strategies in a MRSA-PVL model.

Treatment	PVL– strain			PVL+ strain		
	Bacterial lung concentration	% early mortality	PVL assesment	Bacterial lung concentration	% early mortality	PVL assesment
Control	8.75±0.59	66%	–	8.07±1.30	60%	0.266±0.504
VA	8.05±0.58	75%	–	6.36±2.45	50%	0.177±0.267
CLOX	1±0	0%	–	1±0	0%	0.164±0.120
LZO	3.05±1.32	50%	–	4.02±1.80	50%	0.042±0.024
CLI	1±0	0%	–	1±0	0%	0±0

O373 The colonizing ability of *E. coli* strains isolated from patients with inflammatory bowel disease

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Objectives: *E. coli* strains of the phylogenetic group B2 were isolated more frequently from patients with IBD compared to healthy controls. Furthermore, B2 strains with ExPEC genes were found more frequently among IBD patients with active disease compared to patients with inactive disease. It is of great interest to further characterise those strains. The objective was to investigate the colonising traits of B2 *E. coli* strains isolated from patients with IBD in a gastrointestinal animal model.
Methods: Two B2 *E. coli* strains from IBD patients and one *E. coli* isolate from a healthy person were investigated. Strains were also compared to the probiotic *E. coli* Nissle 1917 and to the laboratory *E. coli* MG1655. Growth rate was determined *in vitro*. The adhesion capacity was assessed in the human epithelial intestine cell line (Int-407). The colonising ability was tested by colonisation of the large intestine of streptomycin treated mice. Different colonisation, competition and inoculation procedures were tested in this model.
Results: Both IBD strains had increased growth rate compared to the *E. coli* from the healthy person and the *E. coli* Nissle strain. Likewise, the IBD strains had an increased adhesion capacity in the cell adhesion assay. In the animal model all strains were able to colonise the gastrointestinal tract in high levels when inoculated alone. In competition with the probiotic Nissle strain both IBD strains co-colonised, though to a log higher (in CFU/g faeces) than Nissle, when inoculated in equal high levels. When *E. coli* Nissle was inoculated in high levels compared to IBD strain in low levels the strains also co-colonised at the end of the experiment. The *E. coli* strain from the healthy person also co-colonised with *E. coli* Nissle in intestine of the mice, but stabilised at a log lower than Nissle strain, when inoculated in equal high levels and two logs lower when inoculated at a lower level than *E. coli* Nissle.
Conclusion: IBD strains had increased growth rates and adhesion abilities compared to *E. coli* isolated from a healthy person and *E. coli* Nissle. Furthermore, *E. coli* from IBD patients had an increased ability to co-colonise with the probiotic *E. coli* Nissle strain.

New antibacterial agents

O374 MUT056399, a new drug candidate against *S. aureus*: PK parameters and safety

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Background: MUT056399 is a novel antistaphylococcal agent targeting the essential enzyme FabI. We present here its key properties: *in vivo* efficacy, preclinical pharmacokinetics and safety, finally the pharmacology data of phase I.
Methods: Standard procedures were used for evaluation of PK parameters. Preclinical safety sudies were conducted on mice, rat and dogs in GLP conditions. *In vivo* assays were performed using the mice models of septicaemia and thigh infection using methicillin-susceptible and non susceptible *S. aureus* (MSSA and MRSA). Linezolid and vancomycin were used as comparators.
In Phase I, ascending doses of MUT056399 formulated in HPBCD were administrated to healthy volunteers by iv infusions.
Results: PK studies in 3 species indicated that MUT056399 given iv was rapidly cleared from the blood (T1/2 =3–5 h). The MUT056399 molecule was well distributed among tissues without accumulation.
In the thigh infection model with MRSA strains, after a single sc administration the mean static dose was 143 mg/kg/d in neutropenic mice and 45 mg/kg/d in immunocompetent mice.
In the mouse septicaemia model using a single sc administration of MUT056399, the mean ED50 against MSSA ATCC 29213, was 21.6 mg/kg/day, against MRSA strains the mean ED50 was 31 mg/kg/day and 49.6 mg/kg/day for a GISA strain.
In GLP safety studies in rats and dogs MUT56 399 did not affect CV, CNS or respiratory functions.
In Phase I, the administration of a single intra venous doses from 10 mg/d to 1.2 mg /d was safe.
Conclusion: MUT056399 is a novel highly potent antistaphylococcal agent with a clean safety profile. These properties support MUT056399 as a very promising candidate for a novel drug to treat severe staphylococcal infections.

O375 Efficacy of two novel antimicrobials, BC-3781 and BC-3205, in a murine MRSA-pneumonia model

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Background: BC-3781 and BC-3205 are two new antimicrobial agents of the pleuromutilin class, which are both in an early stage of clinical development for intravenous and/or oral treatment of skin and skin structure infections (SSSI) and community-acquired pneumonia (CAP). BC-3781 and BC-3205 exhibit excellent antimicrobial activity against a range of relevant bacteria frequently identified in SSSI and CAP including methicillin-resistant *Staphylococcus aureus* (MRSA). Invasive severe infections caused by MRSA, particularly those involving persistent bacteraemia and necrotizing pneumonia are associated with high mortality. Since treatment with currently available antibiotics is often unsatisfactory novel antibacterials with improved efficacy against severe MRSA infections are urgently needed.
Methods: Murine pulmonary infections caused by MRSA were established in neutropenic female BALB/c mice. Mice were grouped (n = 6) and subcutaneous therapy using BC-3781, BC-3205, linezolid or vancomycin started 2 h post infection (1.5×10^7) with a single dose and continued for two days with a bid dosing regimen. Lungs of mice were dissected on day 3 after start of infection. The bacterial burden in pulmonary tissues was determined using standard plating techniques. Untreated animals died on day 2. An Emax dose response model was used to obtain the bacteriostatic dose levels and the maximum killing potency of all tested compounds.
Results: BC-3781 and BC-3205 showed excellent efficacy with a reduction of 3–4 log10 CFU/lung being achieved with doses of 160 mg/kg BC-3205 and BC-3781, respectively. For the same reduction of viable MRSA in lungs a dose of 240 mg/kg/day linezolid was required. The maximum killing potency of vancomycin was only ~1.3 log10 CFU/lung at a dose of 480 mg. BC-3781 and BC-3205 ensured 100% survival already at daily doses greater than 20 and 40 mg/kg, respectively. Doses of >60 mg/kg/day linezolid and >240 mg/kg/day vancomycin, were needed for 100% survival.
Conclusions: Both compounds, BC-3205 and BC-3781, demonstrated excellent efficacy in a murine pulmonary infection caused by MRSA. Both compounds, BC-3781 and BC-3205, showed superior efficacy compared to the standard of care antibiotics linezolid and vancomycin.

O376 BAL30072, a new sulfactam with excellent *in vitro* and *in vivo* activity against *Escherichia coli* producing extended-spectrum β-lactamases

S. Navon-Venezia, O. Hermesh, B. Kuzmenko, M. Page, Y. Carmeli (Tel Aviv, IL; Basel, CH)*

Objectives: BAL30072 is a novel siderophore monocyclic β-lactam belonging to the sulfactams. We evaluated the *in vitro* antimicrobial activity of this compound against a panel of *Escherichia coli* clinical isolates and studied its efficacy against peritonitis and sepsis in mice caused by an ESBL-producing strain of *E. coli*.
Methods: The antimicrobial activity of BAL30072 was tested against 39 strains (20 wild type and 19 ESBL-producers). MICs were determined using agar dilution on Mueller-Hinton agar supplemented with 2, 2' bipyridyl to induce iron transport. *In vivo* efficacy was evaluated against a CTX-M-15-like-producing *E. coli* strain using a lethal peritonitis sepsis model in neutropenic ICR mice. Survival was monitored for seven days after infection. *E. coli* inocula ranged from 2.5×10^6 to 5.0×10^6 cfu/mouse (2–5 X LD50) and BAL30072 was administered intraperitoneally 3 times daily for 3 days (doses of 100 mg/kg down to 12.5 mg/kg) following infection. Saline was administered to the untreated control group and meropenem was administered to the treated control group.
Results: MIC50/MIC90 of BAL30072 for wild type, and ESBL-producing *E. coli* were 0.125/0.5, and <0.0625/1 mg/L, respectively (ranges <0.0625–1 and <0.0625–256 mg/L, respectively). The MIC of BAL30072 against the ESBL-*E. coli* used in the mouse model was <0.0625 mg/L. *In vivo* experiments in mice infected intraperitoneally with various *E. coli* inocula showed that, at doses from 100 mg/kg down to 12.5 mg/kg, BAL30072 conferred protection similar to meropenem (90–100% survival). Survival in the placebo group was 0–33%.
Conclusion: BAL30072 is an effective antibacterial agent against clinical isolates of *E. coli*, including ESBL-producing strains. It is highly effective treatment against infection caused by ESBL-producing *E. coli* as demonstrated in mice peritonitis-sepsis model.

O377 Activity of ACHN-490 and other aminoglycosides vs. carbapenem-resistant Enterobacteriaceae isolated in the United Kingdom

S. Mushtaq, M. Warner, J-C. Zhang, S. Maharjan, M. Doumith, N. Woodford, D. Livermore (London, UK)*

Objectives: ACHN-490 is a novel sisomicin derivative stable to nearly all aminoglycoside-modifying enzymes. We compared its activity with that of other aminoglycosides vs. clinical Enterobacteriaceae representing the diversity of carbapenem resistance types now emerging in the UK. Most were multiresistant.
Methods: The 81 isolates comprised those with the Class A carbapenemases KPC (n = 10) or SME-1 (1); Class B enzymes, IMP (13), VIM (5) and NDM (17) and the Class D enzyme, OXA-48 (19) or with combinations of impermeability plus an AmpC or ESBL (16). They included 52 *Klebsiella* spp., 18 *Enterobacter* spp., 6 *E. coli* and 5 others. Carbapenemase genes were identified by PCR and sequencing as were those encoding the 16S rRNA methylases ArmA and RmtA-C. MICs were measured by CLSI agar dilution.

Results: ACHN-490 was active against all 64 non-NDM+ isolates at <2 mg/L, with 95% of MICs between 0.06 and 0.5 mg/L. Isepamicin was active at 8 mg/L against 62 of these 64 whereas 35%, 61% and 19% were resistant to gentamicin at 4 mg/L, tobramycin at 4 mg/L and amikacin at 16 mg/L, respectively. Among the 17 isolates with the NDM-1 enzyme – many from patients with prior medical contact on the Indian subcontinent – 16 harboured armA or rmtC and were resistant to ACHN-490 and to all other human-use aminoglycosides at >32 mg/L; armA and rmtC were absent from the sole ACHN-490-susceptible NDM-1-positive strain. Apramycin, a veterinary analogue, was active at 4–8 mg/L against (i) control strains, (ii) the NDM-1-positive isolates with armA or rmtC and (iii) all other strains except a *Serratia* with SME-1 enzyme (MIC > 128 mg/L).
Conclusion: ACHN-490 had potent activity vs. all the carbapenem-resistant isolates screened except those with combinations of NDM-1 enzyme and ArmA or RmtC rRNA methylases; the international prevalence of these enzymes needs urgent surveillance. Evasion of ArmA and RmtC by apramycin is striking and may facilitate future human drug development.

O378 Novel antimicrobial agent NI02 displays potent activity against a range of pathogenic bacteria

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Objectives: The ever increasing resistance of pathogenic bacteria towards the current arsenal of antimicrobial therapeutics emphasizes the urgent requirement for development of novel agents. It is currently estimated over 80% of clinical infections are also biofilm associated, therefore, agents that are effective against multiply resistant, biofilm forming would be extremely advantageous. The aim of the current study was to assess the effect of novel antimicrobial agent (NI02) against a range of pathogenic bacteria and against a clinical isolate *Staphylococcus epidermidis* that displays methicillin resistance and a biofilm phenotype.
Methods: The deferred antagonism method was used to assess the range of activity of NI02 against a selection of pathogenic Gram-positive and Gram-negative bacteria. The traditional biofilm forming assay was also employed using preparations of NI02 with an inhibitory activity of >2560 AU/ml to coat microtitre wells and also to treat mature (48 hour) biofilms.
Results: The deferred antagonism assay showed NI02 to have good activity against a range of Gram-positive pathogens including vancomycin resistant enterococci, methicillin resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Listeria* sp., *Bacillus* subtillis and *Bacillus cereus* and Gram-negative isolate *Moraxella catarrhalis*. NI02 also showed good activity against *Staphylococcus epidermidis* biofilms as coating microtitre plate wells resulted in complete inhibition of biofilm development and treatment of mature biofilms resulted in a significant reduction in biofilm density ($P \leqslant 0.001$).
Conclusion: The results obtained in the current study demonstrate the broad-spectrum activity of antimicrobial agent NI02 against a range of pathogenic bacteria and the ability of the inhibitor to prevent and disrupt biofilms.

Carbapenemases other than KPCs: MBL and OXAs

S380 Current epidemiology of MBL-producing Gram-negative non-fermenters and Enterobacteriaceae

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Gram-negative bacteria producing acquired metallo-β-lactamases (MBLs) represent one of the greatest challenges for modern antimicrobial therapy. MBL producers commonly exhibit resistance not only to a broad-spectrum of β-lactams but also to a variety of non-β-lactam agents owing to association of MBL genes with those affecting different groups of antibiotics. The IMP and VIM enzymes are the most common acquired MBLs. These enzymes were first reported in Japan and Europe but are now broadly disseminated in many countries of all populated continents. Production of acquired MBLs is most often observed in *P. aeruginosa*, with VIM-2 being the most ubiquitous enzyme, and less frequently in *Acinetobacter* spp. and in Enterobacteriaceae, mainly in *Klebsiella pneumoniae* and *Enterobacter cloacae*, with VIM-1 or VIM-4 being the most typical variants in the latter species. MBLs of other genetic groups were either identified in isolates causing sporadic infections and local outbreaks (e.g. GIM-1, SIM-1, KHM-1, AIM-1) or as endemic types in some geographic areas (e.g. SPM-1 in Latin America). However, the recent reports of the international importation of SPM-1 from Brazil to Switzerland and of NDM-1 from India to the UK provide continuing evidence of the increasing complexity of MBL epidemiology. The prevalence of MBL-producers varies significantly among different countries and their epidemiology now ranges from sporadic monoclonal outbreaks to polyclonal endemicity. Furthermore, MBL-producing strains are no longer confined to nosocomial environment but are being identified also in residents of long-term-care facilities and even in patients with community-acquired infections. The association of MBL genes with mobile genetic platforms (e.g. Tn402-like transposons in the case of blaVIM genes), broad host range plasmids and highly epidemic clones (e.g. *P. aeruginosa* clonal complexes CC235 and CC111) were apparently implicated in the rapid spread of these important resistance determinants.

S381 Natural and acquired CHDLs: biochemical properties, expression, and impact on resistance

L. Poirel (Le Kremlin Bicetre, FR)*

Class D β-lactamase-mediated resistance to β-lactams has been increasingly reported during the last decade. Those enzymes also known as oxacillinases or OXAs are widely distributed among Gram negatives. They do usually possess a narrow spectrum β-lactam hydrolysis profile, but some of them do possess the ability to hydrolyse carbapenems, even at a low level. They have been named Carbapenem-Hydrolysing class D β-Lactamases, or CHDLs.
CHDLs are known to be intrinsic in some Gram negative rods, including *Acinetobacter baumannii* (OXA-51-like enzymes) and *Pseudomonas aeruginosa* (OXA-50-like enzymes), but usually play a minor role in the natural resistance phenotypes. Besides those naturally-occurring CHDLs, others have been identified as acquired. They have been mostly identified in *Acinetobacter* spp. (mostly in *A. baumannii*), and belong to four distinct groups according to their amino acid sequences, namely the OXA-23, OXA-40, OXA-58, and OXA-143 groups. The genes encoding those CHDLs in *A. baumannii* are either plasmid- or chromosome-encoded. Interestingly, the blaOXA-23 progenitor was shown to be *Acinetobacter* radioresistens, a non-pathogenic and environmental species.
Despite their weak ability to hydrolyse carbapenems, those CHDLs are responsible for high level resistance to those compounds when their expression is associated with other mechanisms such as efflux overepression, penicillin-binding protein modifications, or porin loss. Another CHDL, OXA-48, distantly related from those CHDLs identified in *A. baumannii*, has been identified in several enterobacterial species, and mostly in *Klebsiella pneumoniae*. The blaOXA-48 gene which has been found to originate from Shewanella spp. is plasmid-mediated, increasingly reported in different countries, and responsible for resistance to carbapenems when associated with permeability deffects.
Acquired CHDL encoding genes have been mostly identified in association with insertion sequences that are either responsible for their expression and their acquisition. In particular, both the blaOXA-48 and blaOXA-23 genes have been identified inside composite integron structures (IS1999- and ISAba1-made, respectively).

S382 Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii*: genes, plasmids and clones

N. Woodford (London, UK)*

Carbapenems (excluding ertapenem) are treatments of choice for infections caused by multi-resistant *A. baumannii* so the emergence of carbapenem resistance is a serious problem. As with other Gram-negative species, carbapenem resistance has been attributed to multiple mechanisms, including reduced permeability through loss of outer membrane proteins, altered penicillin-binding proteins and over-expression of weakly carbapenem-hydrolyzing β-lactamases, but the major mechanism is carbapenemase production. Diverse carbapenemases have been reported in *A. baumannii*, differing in their regional prevalence: isolates with IMP or VIM metallo-enzymes are globally scattered; those with SIM-1 are associated particularly with the Far East, while NDM-1 has been observed only rarely. OXA class D enzymes are the dominant carbapenemases in *A. baumannii*, with 5 phylogenetically distinct groups identified. OXA-51-like enzymes are intrinsic to the species, and are only associated with carbapenem resistance when up-regulated by an adjacent insertion sequence, usually ISAba1. By contrast, OXA-23-like, OXA-40-like, OXA-58-like and OXA-143 carbapenemases have all been acquired via horizontal gene spread. These enzymes usually confer clinically significant carbapenem resistance, although the MICs for individual isolates are influenced by the particular gene present, its copy number, and presence of accessory mechanisms (e.g. loss of the CarO porin). The blaOXA genes may be located on the chromosome, as part of large resistance islands, or on plasmids. Local outbreaks of carbapenem-resistant *A. baumannii* typically include a predominant strain, and strains from epidemiologically unlinked sites may show sufficient similarity to be regarded as the same clone. There are two dominant carbapenem-resistant clones in the UK, 'OXA-23 clone 1' has OXA-23 enzyme, and the 'South-east clone' has ISAba1-up-regulated OXA-83 enzyme; both have affected >50 hospitals and belong to sequence group 1 (based on ompA, csuE and blaOXA-51-like alleles), which is European clone II. In other countries there may be greater evidence for horizontal gene spread in addition to strain spread, as observed for OXA-72 (an OXA-40-like enzyme) in a Taiwanese hospital. There are few therapeutic options for infections caused by carbapenem-resistant strains other than a polymyxin or glycylcycline and effective infection control remains essential to control their spread and impact.

Tuberculosis at the cutting edge

S383 New insights into host–pathogen responses in TB

A. Lalvani (London, UK)*

Host–pathogen interactions determine the outcomes *M. tuberculosis* infection by influencing key control points in the natural history of infection, including progression from latent infection to active disease and the clinical spectrum of active disease itself.

Our understanding of the natural history and epidemiology of tuberculosis infection are based on two key century-old tools, the tuberculin skin test and chest radiography. The advent of new, more accurate markers of infection presents an opportunity to re-evaluate our conventional understanding of the host–pathogen relationship in tuberculosis, including the natural history and epidemiology of tuberculosis infection.

The application of interferon-gamma release assays in epidemiologically well-defined tuberculosis outbreaks and longitudinal cohorts points towards a new class of host–pathogen interaction in a subset of tuberculosis contacts that likely involves a transient, self-resolving infection. This entity was not previously appreciated and has significant implications for our understanding of the host–pathogen interaction in tuberculosis infection which will be discussed. T cell-based testing for tuberculosis infection has also uncovered a role for BCG vaccine in preventing infection despite *M. tuberculosis* exposure. The implications of this phenomenon for the design and evaluation of new tuberculosis vaccines, as well from tuberculosis control, will be discussed.

Newer biomarkers, measuring multiple facets of the host response to tuberculosis infection and disease, have recently been developed and may enable discrimination between the several stages in the natural history of tuberculosis infection, eg differentiating between active and latent tuberculosis infection. These new types of biomarkers will be reviewed as well as the the new insights they could provide for our understanding of the host–pathogen equilibrium in tuberculosis infection.

S385 Genomics and therapeutics

S. Cole (Lausanne, CH)*

New medicines for tuberculosis (NM4TB): Tuberculosis (TB) is one of the oldest infectious diseases known to man and has infected one third of the world's population. As a result, someone dies from the disease every 15 seconds and over 20 million more people will lose their lives to TB in the next decade. Although directly observed short course chemotherapy (DOTS) is available to treat the disease, this treatment is old, slow and inefficient by the current standards of the pharmaceutical industry. Furthermore, the efficacy of the DOTS programme has been severely compromised in certain regions by the emergence of multidrug- and extensively drug-resistant strains of *Mycobacterium tuberculosis*. This new menace is of particular concern to Europe and the countries of the former Soviet Union.

In an integrated approach to discover new TB drugs, we have been pursuing a new class of antimycobacterial compounds known as the benzothiazinones (1,3-benzothiazin-4-ones, BTZs). The BTZ kill *M. tuberculosis in vitro*, *ex vivo*, and in mouse models of TB and are exceptionally potent. Using genetics and biochemistry, we identified the enzyme decaprenylphosphoryl-β-D-ribose 2'-epimerase as a major BTZ target. The inhibition of this critical enzyme activity abolishes the formation of decaprenylphosphoryl arabinose, a key precursor that is required for the synthesis of the cell-wall arabinans, thereby provoking cell lysis and bacterial death. The most advanced compound, BTZ043, which is currently in the late preclinical stage, is a candidate for inclusion in combination therapies for both drug-sensitive and extensively drug-resistant TB.

Use of epidemiological methods in making rational treatment decisions in infectious diseases

S389 From case reports to causality: how can we quantify rare adverse events, how should they be considered in the treatment decisions?

R. Platt (Boston, US)*

Rare adverse events after drugs, vaccines, and other therapeutic agents are usually identified after the product is licensed and in widespread use. Historically, surveillance for these has relied on case reports. While case reports have identified many important problems, they have drawbacks, including unknown sensitivity for detecting events of interest and inability to provide the quantitative estimates of risk that are needed to make informed decisions about the balance of benefits and risk.

Quantitative information about risks and also about benefits is becoming increasingly available through the secondary use of electronic health data – both electronic medical records and administrative data – collected during the routine delivery of health care. Among the earliest examples is the U.S. Centers for Disease Control's Vaccine Safety Datalink, which performs both active, near real-time, surveillance for vaccine-associated adverse events, and retrospective epidemiological studies. These methods are being modified to work in groups of health plans with tens of millions of members, and to address the sequelae of exposures to drugs as well as vaccines. An important feature of these methods is that they require minimal sharing of confidential personal

information, because there is no need to create large pooled datasets in order to identify signals of adverse events.
The presentation will illustrate these issues using the current experience evaluating the safety of the H1N1 influenza vaccine and others. It will also address the U.S. Food and Drug Administration's plan to create a Sentinel Network, which will use information from electronic medical records covering 100 million people to perform routine safety evaluation of marketed medical products. Additional considerations regarding assessment of benefit will be addressed.

MRSA bacteraemia and endocarditis – treatment considerations

S392 Pharmacodynamics of anti-staphylococcal antibiotics – implications for clinical management

M. Rybak (Detroit, US)*

MRSA bacteremia and endocarditis represent some of the most challenging and difficult infections to overcome with antibiotics. Understanding the relationships between antibiotic pharmacokinetics and pharmacodynamics, target organism, site of infection and the potential for resistance development is key for successful patient outcome. Optimizing therapy exploits the specific antibiotic pharmacodynamic parameter that best predicts *in vivo* efficacy. For example, in the case of β-lactams, improving the time above the MIC for the targeted pathogen may be accomplished by prolonging the infusion time or utilizing continuous infusion administration. For aminoglycosides, use of high-dose once-daily therapy should increase the Cmax/MIC. Recently, based on the need to improve the performance of vancomycin, higher troughs have been recommended. Higher troughs are needed in order to achieve a higher AUC/MIC ratio which is thought to be important for serious infections like complicated bacteremia, infective endocarditis and pneumonia. Additional factors that affect antibiotic performance include the ability to reach the site of infection (barriers to penetration) and conditions at the site of infection such as pH, protein binding and inoculum. This lecture will discuss specific antibiotic PK/PD characteristics, conditions at the site of infection that diminish antibiotic activity and opportunity to improve performance.

Surveillance of ESBL

O395 Massive emergence of multidrug-resistant Enterobacteriaceae in blood culture isolates of children in Ghana

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Objective: To evaluate the prevalence of antimicrobial resistance in Enterobacteriaceae isolated from blood cultures of children in rural Ghana.
Methods: Between October 2007 and June 2009 blood cultures were collected from children admitted to the Agogo Presbytarian Hospital located in the rural area of the Ashanti – Akim North District with a population of about 135,000. Peds Plus™ bottles and the BacTec™ 9050 system were used and detected strains were identified by Gram staining, selective subcultures, biochemical testing and specific agglutination tests. Minimum inhibitory concentration (MIC) of isolated Enterobacteriaceae and production of extended-spectrum-β-lactamases (ESBL) was determined by the Etest™ method.
Results: In this study isolated Enterobacteriaceae (n = 178) were non-typhoid *Salmonella enterica* (NTS) (113), *Salmonella* Typhi (39), *Escherichia coli* (15) *Klebsiella* sp. (9), *Shigella boydii* (1) and *Enterobacter* sp. (1).
NTS were highly resistant to ampicillin (80.5%), cotrimoxazole (82.3%), chloramphenicol (83.2%), and tetracycline (10.6%). No strain was resistant to ciprofloxacin, but one strain had reduced susceptibility to ceftriaxone. The majority of S. Typhi strains were also resistant to ampicillin (69.2%), cotrimoxazole (60.0%), chloramphenicol (66.7%), and tetracycline (60.0%), but all were completely susceptible to ciprofloxacin and ceftriaxone. All *E. coli* isolates were resistant to ampicillin and 20% produced ESBL. Resistance to cotrimoxacole (93.3%), tetracycline (86.7%), chloramphenicol (53.3%) and ciprofloxacin (26.7%) was frequently detected. All *Klebsiella* strains were resistant to ampicillin and tetracycline and 77.8% produced ESBL. Only one strain was susceptible to cotrimoxazole, 77.8% were resistant to chloramphenicol and one third to ciprofloxacin. The *Enterobacter* strain procuced ESBL and was only susceptible to ciprofloxacin. The *Shigella boydii* isolate was susceptible to ceftriaxone and ciprofloxacin.
Conclusion: The study indicates that the majority of Enterobacteriaceae causing bacteraemia in children in the study area are resistant to the majority of local available antibiotics. Moreover this massive emergence of multidrug-resistant Enterobacteriaceae and ESBL producing *E. coli* and *Klebsiella* strains in clinical isolates indicates a serious medical and economic burden for African healthcare systems. Surveillance programs and prevention strategies have to be established to face this challenge.

O396 Molecular analysis of extended-spectrum β-lactamase dissemination in *Escherichia coli* within families

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Objectives: The means of transmission of ESBL-producing *E. coli* (ESBLEC) isolates in the community are poorly understood. We previously reported that the prevalence of faecal carriage with ESBLEC was higher in relatives of patients with urinary tract infections (UTI) caused by these organisms.in 54 households The aim of this study was to analyse the molecular relationship of the ESBLEC within families settings and the plasmids identified.
Methods: We studied 72 ESBL-producing *E. coli* isolates (34 CTX-M-14, 31 SHV-12, 3 CTX-M-9, 3 CTX-M-15 and 1 CTX-M-32) from 19 families of patients with UTI caused by ESBLEC where at least other faecal carrier within the family was identified (19 patients with UTI and 23 relatives). All isolates were evaluated by XbaI PFGE; the molecular the relatedness was determined by the UPG method using the Dice similarity index on Fingerprinting 3.0. Phylogenetic groups were assigned by multiplex PCR. Transfers of bla genes were carried out by conjugations experiments and transformation by electroporation into J53 and DH10 *E. coli*, respectively. Plasmids were compared by size using S1 nuclease digestion and by the patterns resulted of southern hybridation of HindIII-digested plasmid DNAs from transformants or transconjugants.
Results: A total of 44 different PFGE patterns were identified distributed among phylogenetic groups A (23%), B1 (34%), D (32%) and B2 (11%). Nine relatives (39%) from 7 families (37%) carried the same pulsotype and ESBL than the patient with UTI in their family. In 10 families (53%), the isolates from different members yielded different pulsotypes harbouring the same enzyme; among them, the ESBL was located in similar RFLP plasmidic patterns in 5 families (3 with CTX-M14 and 2 with SHV-12). The CTX-M14 bla gene hybridazed with a single 20 kb hybridation band in 9 plasmids, whereas SHV-12 bla gene was located in fragments with different sizes in 13 plasmids.
Conclusions: Common source or horizontal transmission of the same ESBL-producing strain or plasmid was found in 63% of the families with ESBL-producers carriers. The acquisition of different clones producing different ESBL enzymes by members of the same household was less frequent in our study, but denote other routes of spread in the community.

O397 High frequency of faecal colonization with ESBL-producing Enterobacteriaceae among Swedish persons after travelling outside the Scandinavian countries

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Objectives: Risk factors for acquisition of ESBL-producing Enterobacteriaceae (ESBL-PE) in a low endemic country like Sweden are not well understood. Previous studies have shown that travel might increase the risk. The aim was to study weather travel to countries outside Scandinavia increases the risk to be colonised by ESBL-PE.
Methods: From Sept 1, 2008 to March 31, 2009 persons attending vaccination clinics in southeast Sweden planning to travel outside Scandinavia for <3 months, were asked to participate. Faecal samples and questionnaires were collected before and after travel. Faecal samples were cultured on chromogenic media: chromID ESBL (bioMérieux) and chromogenic UTI-medium (Oxoid) with discs containing cefotaxime, ceftazidime, cefepime, piperacillin/tazobactam, meropenem and linezolid. Isolates with suspected ESBL-phenotype were subcultured and confirmed according to the methods of the extended ESBL definition suggested by the Swedish Reference group for Antibiotics (SRGA) (www.srga.org).
Results: Of 262 enrolled persons, 231 submitted faecal samples and questionnaires before and after travel and were included for analysis. Before and after travel, 9 (4%) and 73 (32%) persons were colonised by ESBL-PE, respectively. No KPC were found. *E. coli* was the most commonly found species. Gender, age, use of oral cholera vaccination or antibiotics and duration of travel were similar among persons acquiring ESBL-PE during travel (travel associated (TA)-carriers, n = 69) and persons never colonised by ESBL-PE (non-carriers, n = 154). TA-carriers more often reported diahorrea (54%) or other abdominal symptoms (25%) during travel, compared to non-carriers (38% and 13%). Business was a more common cause for travelling among TA-carriers (12%) than non-carriers(6%), whereas TA-carriers more often reported travelling as "backpackers", 16% vs. 8%. The frequencies of TA-carriers after travel to the most visited countries (>10 travellers/country) were: India 82%, Egypt 57%, Peru 36%, Thailand 37%, South Africa 27% and Tanzania 24%, respectively.
Conclusions: Travel increases the risk of faecal colonisation by ESBL-PE. Acquisition of ESBL-PE during travel is associated with abdominal symptoms such as diahorrea.

O398 Comparative epidemiology of faecal carriage of extended-spectrum β-lactamase-producing enterobacteria in 2 hospitals specialized in liver diseases in Egypt and in France

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Objectives: Extended-spectrum β-lactamase (ESBL) is a worldwide public health problem. Few data are available on ESBL in Egypt. The aim of this study was to compare the fecal carriage of ESBL-producing enterobacteria (ESBL-E) in patients admitted to the hepatology department of 2 hospitals specialized in liver diseases, Theodor. Bilharz Research Institute (TBRI) in Cairo (Egypt) and Beaujon hospital (Bj) in Clichy (France).
Methods: All patients admitted during May-June 2009 for more than 24 h in the hepatology department in TBRI and in Bj were screened at admission with a rectal swab for detection of ESBL-E digestive carriage. CTX-M groups were identified by PCR and TEM and SHV derivatives with the check point system. Phylogenetic groups of *E. coli* were determined by multiplex PCR and clone ST131 by PCR of gene pabB.The following demographic and clinical data were collected: gender, age, previous hospitalizations, underlying diseases and ascites.
Results: During the study period, the prevalence of patients with ESBL-E was 77.6% (45/58) in TBRI and 6.5% (13/199) in Bj. A previous hospitalization was more common (p = 0.003) in Bj patients (93%) than in TBRI patients (45%). The 2 populations also differed with regard to the underlying diseases (p = 0.0003). We studied 49 ESBL-E from TBRI and 14 from Bj because 4 and 1 patients, respectively, had 2 ESBL-E. Of the 49 ESBL-E of TBRI, 90%, 8% and 2% were *E. coli*, *K. pneumoniae*, and *E. cloacae,* respectively. Of the 14 ESBL-E from Bj, 72%, 14%, 7% and 7% were *E. coli*, *E. cloacae, K. pneumoniae* and *K. oxytoca,* respectively. The species distribution did not differ between the 2 countries. In TBRI, 59%, 29% and 12% of ESBL were enzymes of groups CTX-M-1, CTX-M-9 and SHV, respectively, whereas in Bj, 79%, 14% and 7% were of groups CTX-M-1, TEM and SHV, respectively. No significant difference was observed for enzymes of groups CTX-M-1 and SHV. Of the 44 TBRI *E. coli*, 59% belonged to phylogenetic group A, 27% to D, 7% to B1 and 7% to B2 whereas of the 10 Bj *E. coli,* 40% belonged to A, 40% to B1 and 20% to D. ESBL-E of group B1 were significantly higher in Bj than in TBRI (p = 0.04). The 3 TBRI B2 isolates belonged to clone ST131.
Conclusions: The prevalence of patients with ESBL-E fecal carriage was very high in TBRI. More than 50% of these patients did not have a previous hospitalisation, suggesting a very high prevalence of ESBL-E in t the community in Egypt.

O399 Risk of acquiring multidrug-resistant Gram-negative bacilli from prior room occupants

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Objectives: Two recent studies identified admission to a room previously occupied by a methicillin-resistant *Staphylococcus aureus* (MRSA) or a vancomycin-resistant enterococci patient as an independent risk factor for acquisition of these bacteria. The aim of our study was to determine whether admission to an ICU room previously occupied by a multidrug-resistant Gram negative bacilli (MDRGNB) carrier increase the risk of acquiring these bacteria by subsequent patients.
Methods: Prospective observational cohort study performed in a 30-bed ICU during a 1-year period. All patients hospitalized >48 h were eligible. Patients with MDRGNB at ICU admission were excluded. MDRGNB were defined as MDR *Pseudomonas aeruginosa* (resistant to imipenem or to ceftazidime), *Acinetobacter baumannii*, and extended spectrum β-lactamase (ESBL) producing GNB. All patients were hospitalized in single rooms. The infection control policy included isolation techniques, routine screening of MDR bacteria (MDRGNB and MRSA), written antibiotic treatment protocol, and continuous surveillance of nosocomial infections. Isolation techniques were used for all patients at ICU admission, until receipt of screening results. In addition, these techniques were used for patients with MDR bacteria, and for all immunosuppressed patients. Routine screening of MDR bacteria (nasal, and anal swabs; and tracheal aspirate in intubated patients) was performed for all patients at ICU admission and weekly thereafter. Risk factors for MDR *P. aeruginosa*, *A. baumannii*, and ESBL were determined using univariate and multivariate analysis.
Results: 511 consecutive patients were included. 82 (16%) patients acquired a MDR *P. aeruginosa*, 57 (11%) an *A. baumannii*, and 50 (9%) an ESBL GNB. Independent risk factors for ICU-acquired *P. aeruginosa* were prior occupant colonized with *P. aeruginosa* (OR[95% CI]=2.3[1.2–4.3], p = 0.012), surgery (1.9[1.1–3.6], p = 0.024), and prior piperacillin/tazobactam use (1.2[1.1–1.3], p = 0.040). Independent risk factors for ICU-acquired *A. baumannii* were prior occupant colonized with *A. baumannii* (4.2[2–8.8], p < 0.001), and mechanical ventilation (9.3[1.1–83], p = 0.045). Independent risk factors for ICU-acquired ESBL were tracheostomy (2.6[1.1–6.5], p = 0.049), and sedation(6.6[1.1–40], p = 0.041.
Conclusion: Admission to an ICU room previously occupied by a MDR *P. aeruginosa* or an *A. baumannii* carrier is an independent risk factor for acquisition of these microorganisms by subsequent room occupants.

O400 Intestinal colonization with Gram-negative bacteria and subsequent ICU-acquired bacteraemia during selective digestive decontamination

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Objectives: In a recent study (NEJM 2009;360:20) selective digestive decontamination (SDD) was, compared to Selective Oropharyngeal Decontamination (SOD), associated with a significant reduction in ICU-acquired bacteremia caused by Gram-negative bacteria (GNB). The difference between SDD and SOD is intestinal tract decontamination and standard 4 days of cefotaxim during SDD (and not during SOD). We hypothesized that intestinal colonization with GNB was associated with subsequent ICU-acquired GNB bacteremia in patients receiving SDD.
Methods: Retrospective cohort study in a tertiary care ICU in the Netherlands, including all patients that had received SDD between Sept 2008 and Sept 2009. Rectal carriage with GNB was determined twice weekly during ICU-stay. For each patient-day the status of intestinal GNB colonization was determined, creating patient-days with and without intestinal GNB colonization. Incidence densities of ICU-acquired GNB bacteremia were calculated for 1,000 patient-days with and without intestinal GNB colonization.
Results: 730 patients (7762 patients-days at risk) were included and 650 (89%) had >1 episode of rectal GNB colonization during ICU-stay. 23 patients (3.2%) had >1 episode of ICU-acquired GNB bacteremia. Incidences of ICU-acquired GNB bacteremia were 6.1 and 1.9 per 1,000 patient days for the periods with and without intestinal GNB colonization [relative risk 3.78 (95% CI 1.66–8.61)]. The median onset of ICU-acquired bacteremia with and without intestinal GNB was 12 (IQR 37.5) and 8.5 (IQR 14.3) respectively (p = 0.4); 3 and 2 ICU-acquired bacteremias occurred within the first 4 days after ICU-admission. 10 (43%) Episodes of bacteremia were preceded by rectal colonization with the same species, being most frequently *Serratia* spp (n = 3) and *Pseudomonas aeruginosa* (n = 3). 3 patients had already lost rectal carriage at the time of bacteremia with the same species.
Conclusion: Intestinal colonization with GNB during SDD is associated with an almost 4-fold risk of developing GNB bacteremia. 78% of ICU-acquired bacteremia occurred after day 4, indicating that this association seems not to be influenced by cefotaxime use.

O401 Characterization of CTX-M15-producing *Klebsiella pneumoniae* associated with an outbreak in a neonatal intensive care unit. A follow-up study shows long-term faecal colonization and transmission to family members

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Objective: Molecular characterization of a multiresistant extended-spectrum βlactamase (ESBL)-producing *Klebsiella pneumoniae*-strain associated with an outbreak in a neonatal intensive care unit (NICU) at Stavanger University Hospital, Norway. Evaluate long term colonization in affected newborns and transmission to family members.
Methods: Rectal/fecal samples were cultured on modified MacConkey agar and on chrom ID ESBL-plates (bioMérieux). Susceptibility testing was performed by disk diffusion and Etest. ESBL-production was confirmed by double disk approximation test. βlactamase-genes were detected by PCR and typed by DNA sequencing. Clonal relatedness was examined by PFGE. A follow-up study has been initiated. Monthly rectal/fecal samples are collected from the newborns and their families.
Results: The outbreak was disclosed Jan 30th 2009 after identification of multiresistant *K. pneumoniae* in clinical samples from three patients. Subseqently, 58 children were found to be colonized with ESBL-producing *K. pneumoniae* after screening all patients in the NICU (26/27 colonized) and children discharged from the NICU or maternity ward during Nov 2008 to Jan 2009 (28/500 colonized). After cohorting and strengthened infection control measures another four children (4/89) at the NICU were colonized during Feb to April. Only one patient had a severe clinical infection (septicaemia) and recovered successfully after treatment with meropenem. All isolates (n = 58) expressed resistance to aztreonam, 3rd and 4th generation cephalosporins, clavulanic acid synergy as well as co-resistance to aminoglycocides, trimethoprim-sulfamethoxazole and nitrofurantoin. CTX-M-type (group1) was confirmed by PCR and DNA sequencing. All isolates examined (n = 17) displayed an indistinguishable Xba1-PFGE-pattern. 49 colonized children and 80 family members were included in the follow-up study. After nine months of follow-up, 45 children (92%) are still colonized. Transmission to 16 family members (20%) has been detected.
Conclusions: This is the first major hospital outbreak caused by ESBL-producing Enterobacteriaceae in Norway. The outbreak onset was about two months before the ESBL-producing *K. pneumoniae*-strain was identified. A subclinical outbreak like this is difficult to detect unless patients are regularly screened. Long time carriage in newborns is a possible reservoar for the spread of ESBL-producing bacteria in hospitals and in the community.

O402 Duration of extended-spectrum β-lactamases colonization during hospitalization and after discharge

M. Leverstein-van Hall, J. Muilwijk (Bilthoven, NL)*

Objectives: Infection control practices on extended spectrum β-lactamase (ESBL) include barrier precautions and flagging of carriers. The Dutch guidelines on infection control of ESBLs recommend to perform twice-weekly surveillance cultures and to end the precautions if 2 consecutive cultures are negative. The cost-effectiveness of these cultures is dependent on the proportion of pts that remain positive. These data are, however not available. In addition, carriers should be flagged. However, there are little data on the duration of ESBL colonization and therefore recommendations for the duration of flagging are lacking. The aim of this study was 1) to determine the duration of ESBL colonization, both hospital and community acquired and 2) to determine the proportion of pts that remain positive during hospitalisation.
Methods: In the 2008 database of the national antimicrobial resistance surveillance system (ISIS-AR) each pt with a positive culture with either *Escherichia coli* (EC) or *Klebsiella pneumoniae* (KP) (intermediate) resistant (I/R) to third generation cephalosporines (CEPH) and at least one follow up culture with an EC or KP were identified. For each pt the time period was determined between the first ESBL positive and first negative culture. The primary end point was July 31st, 2009. The duration of colonisation was estimated by Kaplan–Meier survival analysis, in which all pts without a last negative culture were censored.

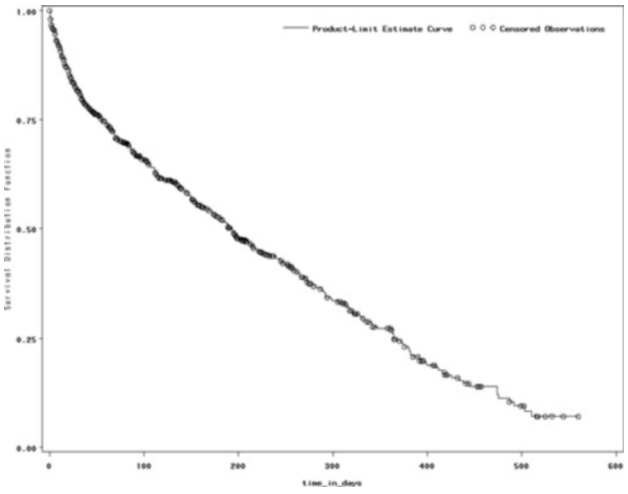

Figure. Days to 3rd gen. cephalosporin susceptible isolate.

Results: In 2008, 1139 pts had a positive culture with an isolate I/R to CEPH (ESBL confirmation test positive in 81% of pts). 926 pts had an

ESBL positive follow-up culture. The median colonisation time was 192 days (95% CI 172–216), the first quartile 56 days (95% CI 41–69) and the third quartile 365 days (95% CI 334–391) (Figure).
For 575 pts the first positive isolate was cultured during admission. Of the 470 with a follow up culture the proportion of pts with a positive isolate after week 1 was 63% and after week 2 58%.
Conclusions: At least 75% of pts remain ESBL positive for at least 2 months and 25% of the pts is still carrier after one year. This implicates that flagging should be continued until screening cultures at (re-) admission are shown to be negative.
At least 60% of the patients remain ESBL positive during hospitalisation. Since the real percentage is likely higher due to interference of antimicrobial therapy with culture results, the advice to perform 2-weekly surveillance cultures should be reconsidered.

O403 Individual risk factors for colonization with carbapenem-resistant *Klebsiella pneumoniae* among residents in post-acute-care facilities in Israel: a matched case–control study

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Background: Since 2006, a nationwide outbreak of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has been reported in acute-care hospitals in Israel. In a point-prevalence study in post-acute-care facilities (PACFs), we previously identified type of ward, prolonged length of stay (LOS) and infection control policies as ecological factors associated with CRKP colonization. It is important to distinguish between ecological and individual-level risk factors in order to direct interventions appropriately. Here we extended our analysis to assess individual risk factors for colonization among residents without history of CRKP carriage.
Methods: A point-prevalence study was conducted in 12 PACFs. Rectal swabs were obtained from residents without a history of CRKP carriage in 33 wards. Suspicious colonies growing on CHROMagar™ KPC plates were speciated and tested for carbapenem susceptibilities by VITEK® 2, supplemented by Etest®. We used a nested, matched case–control study design to assess individual risk factors for colonization. Cases were defined as patients with positive rectal screening. Controls were selected from patients with negative rectal screening, matched to cases by ward. Patient data collected from medical records included demographic characteristics, comorbid conditions, presence of skin lesions, presence of invasive devices, antibiotic exposure, number of colonized roommates, and PACF LOS.
Results: Of 1004 residents without history of CRKP, colonization was detected in 119 (12%). Covariates entered into the matched multivariable model included: Norton score, antibiotic exposure during the past 3 months, skin lesions, receipt of amoxicillin–clavulanate during the past month and colonization with other resistant pathogens. Independent risk factors for CRKP colonization were: antibiotic exposure during the past 3 months (OR 1.6, 95% CI 1.03–2.6, p = 0.04) and colonization with other resistant pathogens (OR 1.6, 95% CI 1.06–2.6, p = 0.03).
Conclusion: This nested, matched case–control study identified an important modifiable risk factor for CRKP colonization not identified in the ecological analysis: antibiotic exposure. Antibiotic control programs may have an important role in decreasing the burden of CRKP in PACFs.

O404 Seasonal and ascending trends in the incidence of extended-spectrum β-lactamase-producing *E. coli* and *Klebsiella* sp. in two German university hospitals

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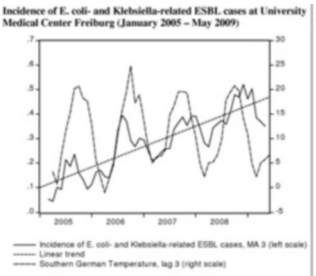
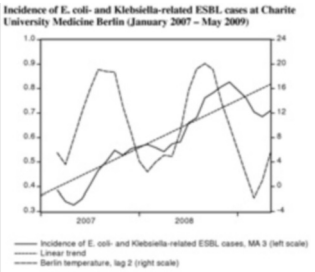
Objectives: In the present study, the incidence of extended spectrum {β}-lactamase (ESBL) producing strains were analysed for general trends and seasonality.
Methods: Monthly data on ESBL producing strains were collected retrospectively at two large university hospitals in the Southwest and Northeast Germany. For the analysis we focussed on *E. coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* for consistency both between the two settings and during the study periods. Multivariate time-series analyses were carried out to explain variations in the monthly incidence densities of ESBL cases (*E. coli*- and/or *Klebsiella*-related ESBL cases per 1000 patient days) in the two settings (Berlin and Freiburg). For the final models, we were able to incorporate variables for the ascending linear trends and other variables representing the average monthly temperature.
Results: Our models show that in general all the incidences of ESBL cases show an increasing trend. In addition, all incidences of ESBL cases responds positively to the average temperature, meaning that in summer when the temperature is high more ESBL cases were detected than during winter months (See Figure for details). The study methodology was also applied to the incidences of MRSA in the two settings, but an association with the average temperature could not be detected.
Conclusions: In the present study, we affirmed that ESBL-producing *E. coli* and *K. pneumoniae* are an emerging problem in German hospitals. Furthermore, we demonstrated that the monthly incidence of ESBL strains is highly correlated with the mean monthly temperature, a fact which should be considered in experimental studies as an additional parameter influencing the incidence of ESBL.



Multivariate model to explain the monthly incidences of E. coli- and Klebsiella-related ESBL cases at University Medical Center Freiburg (R^2=0.67)

Explanatory variable	Lag	Coefficient	T-statistic	P-value
Linear trend	0	0.9771	23.34	<0.001
Temperature	1-4	0.0077	2.81	0.008
Moving average term	3	-0.9627	-35.59	<0.001



Multivariate model to explain the monthly incidences of E. coli- and Klebsiella-related ESBL cases at Charite University Medicine Berlin (R^2=0.88)

Explanatory variable	Lag	Coefficient	T-statistic	P-value
Linear trend	0	1.0739	8.31	<0.001
Temperature	1-4	0.0147	5.02	<0.001
Moving average term	3	0.8995	12.37	<0.001

Invasive candidiasis in ICU

O405 Candidaemia in 536 intensive care units, 2001–2008

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Background: We analyzed the epidemiology and secular trends in candidemia within a network of 536 German ICUs reporting data to the German Surveillance System for Nosocomial Infections.
Methods: CDC standard definitions were used for diagnosis of nosocomial laboratory confirmed primary blood stream infection (BSI). Incidences were calculated by BSI per 100 patients, incidence densities by nosocomial BSI per 1000 patient days and per 1000 central venous catheter (CVC)-days.
Results: 536 ICUs submitted data of 1,942,745 patients, 6,881,170 patient-days and 4,740,019 CVC. Fungi were isolated in 492 of the 6,608 positive BSIs. Of them, 335 BSIs were associated with *C. albicans* and 325 were CVC-associated (97%). *C. albicans* ranked fifth in the most frequent isolated pathogens in BSIs. The mean CVC-associated BSI rate with *C. albicans* stayed stable over time with 0.06 per 1000 CVC-days and it was 0.02 for non-*albicans Candida* (2006–2008). For monomicrobial *C. albicans* BSIs, the mean time from ICU-admission to onset of infection was 14 days. Crude ICU-mortality of all pathogens was highest for *C. albicans* with 19%. Primary BSI differed by type of ICU or hospital and was highest in university hospitals as well as in paediatric, surgical and cardiothoracic ICUs.
Conclusion: In critically ill patients *C. albicans* versus non-*albicans Candida* remained the predominant species in our network of 536 ICUs and ranked fifth of the top pathogens causing primary BSI with a crude mortality of 19%. The mean incidence of primary candidemia with 0.06 *C. albicans* BSI/1000 CVC-days remained unchanged over an eight year period.

O406 Incidence of candidaemia and antifungal sensitivity in critically ill patients

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Introduction: The incidence of invasive candidaemia is increasing all over the world, mainly in critically ill and immunocompromised patients. A shift to non-*albicans* species and a growing resistance to the common antifungal have been noticed over the last years.
Objectives: Aim of our study was to analyze the different *Candida* species isolated from bloodstream infections and the related antifungal susceptibility pattern over a three year period at Policlinico Umberto I of Rome.
Methods: 7574 blood cultures were analyzed from 2007 to 2009 by both automated and manual methods. *Candida* species isolates were identified using chromogenic culture media and by API system (Bio-Merieux). VITEK-2 cards were used to perform the antifungal agents sensitivity to the following drugs: flucytosine, amphotericin B, fluconazole, itraconazole, voriconazole. To determine the susceptibility or resistance patterns, CLSI breakpoints were used.
Results: The overall incidence of invasive candidaemia during the three years under study was 4.75% with a marked increase from 2007 to 2009 (3.85% to 7.5%). The species isolated were the following: *C. albicans* 37.6%, non-*albicans* species 62.4% *(C. krusei* 30.2%, *C. glabrata* 21.7%, *C. parapsilosis* 5.6%, *C. tropicalis* 3.8%, *C. lusitaniae* 1.1%). We observed that the detection of *C. albicans* raised from 7.1% in 2007 to 55.5% in 2009, whereas *C. krusei, C. parapsilosis* and *C. tropicalis* incidence decreased over the three year period. *C. glabrata* isolation was almost the same during the whole period (28.6% in 2007, 22.2% in 2009). As far as *C. albicans* is concerned, we noticed an increase of resistance to amphotericin B (from 0% in 2007 to 6.6% in 2009) and voriconazole (from 0% in 2007 to 13.4% in 2009). *C. krusei* showed a raising resistance to amphotericin B, fluconazole, itraconazole and voriconazole (from 0% to 40%, from 25% to 80%, from 25% to 50%, from 0% to 20%, respectively). In *C. glabrata* a marked increased of resistance to all the antifungal agents was observed. All the isolates of *C. parapsilosis* were resistant to voriconazole; in contrast, *C. tropicalis* was susceptible to all antimycotic agents.
Conclusions: Our study confirms the high incidence of candidaemia in the setting of critically ill patients. *C. albicans* resulted to be the most prevalent species. The overall rate of resistance increased over the study period in all the *Candida* strains under consideration.

O407 Low prevalence of azole-resistant isolates causing fungaemia in patients admitted to intensive care units

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Objectives: The widespread use of azoles may be responsible for an increasing number of episodes of fungemia caused by fluconazole-resistant isolates. We previously showed that, during 2000–2006, only 3.5% of fungemia episodes occurring in patients admitted to intensive care units (ICUs) were caused by a fluconazole-resistant isolate. In the present study, we assess the proportion of episodes of fungemia caused by azole-resistant strains in patients recently admitted to ICUs (2007 to 2009).
Methods: From February 2007 to June 2009, we recorded 80 episodes of fungemia in 78 patients admitted to the ICUs of a tertiary hospital in Madrid, Spain. The distribution of episodes by ICU was as follows: general, 41.2%; neonatology, 32.5%; major heart surgery, 21.3%; and paediatric, 5%. We selected only 1 isolate per episode (83 isolates; 3 patients were co-infected by 2 different species). Antifungal susceptibility to fluconazole and voriconazole was determined using the CLSI M27-A3 microdilution procedure.
Results: The 80 fungemia episodes were caused by *C. albicans* (n = 32; 40%), *C. parapsilosis* (n = 27; 33.8%), *C. glabrata* (n = 9; 11.2%), *C. tropicalis* (n = 6; 7.5%), *C. albicans* + *C. parapsilosis* (n = 3; 3.8%), Arxula adeninivorans (n = 1; 1.2%), Rhodotorula mucilaginosa (n = 1; 1.2%), and Trichosporon inkin (n = 1; 1.2%). The antifungal susceptibility (MIC50/MIC90/MIC range [μg/ml]) of the isolates was 0.25/4/⩽0.125–>128 for fluconazole and ⩽0.015/0.125/⩽0.015–4 for voriconazole. According to the CLSI M27-A3 breakpoints, the isolates were classified as susceptible, (96.2%) and resistant (3.8%) to fluconazole and as susceptible (97.4%), susceptible–dose-dependent (1.3%), and resistant (1.3%) to voriconazole. Three patients had fungemia caused by fluconazole-resistant isolates. The isolates were voriconazole-resistant *(C. glabrata)*, voriconazole-susceptible–dose-dependent *(A. adeninivorans)*, or voriconazole-susceptible *(R. mucilaginosa)*. The 3 patients were admitted to the general ICU *(C. glabrata* and *A. adeninivorans)* and the major heart surgery ICU *(R. mucilaginosa)* and only one had recently received fluconazole. Outcome was favourable in 2/3 patients.
Conclusions: Fungemia caused by fluconazole-resistant isolates occurred only in adult ICUs. Only 3.7% of cases of fungemia were caused by azole-resistant isolates, and this proportion has not increased recently in our hospital. J. Guinea (CP09/00055) is contracted by FIS.

O408 Evaluation of the risk for invasive candidiasis in intensive care patients after cardiothoracic surgery

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Objectives: To improve the diagnostic procedures for invasive *Candida* infections in critically ill patients after cardiothoracic surgery, a prospective surveillance study was performed at our cardiothoracic intensive care unit (ICU) of the Medical University of Vienna.
Methods: Patients admitted to the cardiothoracic ICU for at least 4 days between December 2006 and December 2008 were enrolled into the study. Two times weekly surveillance cultures (n = 4718) for superficial sites were analyzed for the presence of *Candida*. To determine the risk for invasive candidiasis, the following data were assessed: age, sex, Simplified Acute Physiology Score SAPS II, the European System for Cardiac Operative Risk Evaluation (EuroSCORE), length of ICU stay and hospital stay, type and duration of cardiothoracic procedure, number of redo surgeries, additional gastrointestinal (GIT) surgery, parenteral nutrition, severe *Candida* colonization, bacteremia, severe sepsis, leukocytosis or leucopenia and the highest Sequential Organ Failure Assessment (SOFA) Score.
Results: A total of 198 patients were included into the study, 10 ICU patients (5%) developed invasive *Candida* infections. Age, gender, SAPS II and euroSCORE of patients, the type and duration of cardiothoracic procedures and parenteral nutrition were no significant risk factors in our study population. In all invasive *Candida* infection patients severe *Candida* colonization ($p < 0.001$), leukocytosis >14 G/l or leukopenia <4 G/l ($p = 0.03$) and a SOFA score >8 ($p = 0.002$) were found. The risk for invasive *Candida* infections in cardiothoracic ICU patients was calculated using the following determinants: severe *Candida* colonization, leukocytosis or leucopenia, highest SOFA score >8, redo surgical procedures >2 and additional GIT surgery.
Conclusion: Cardiothoracic ICU patients are at risk for invasive *Candida* infections at the presence of severe *Candida* colonization, leukocytosis or leucopenia, a SOFA score >8, additional GIT surgery and when undergoing multiple cardiothoracic interventions >3.

O409 Functional IL-10 and IL-12B polymorphisms are associated with persistent *Candida* spp. bloodstream infection

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Objectives: *Candida* bloodstream infections cause significant morbidity and mortality in hospitalized patients. While clinical and microbiologic factors affecting prognosis have been identified, the impact of immune responses, mediated by cytokines, on outcomes of infection remains to

be studied. The present study assessed the role of genetic variation in cytokine genes on susceptibility and/or clinical outcomes of candidaemia.
Methods: Single nucleotide polymorphisms (SNPs) in six cytokine genes (IFN-γ, IL-10, IL-12B, IL-18, IL-1B, IL-8) and one cytokine receptor gene (IL-12RB) were genotyped and analyzed with logistic regression in 365 patients with candidaemia and 351 non-infected controls. In addition, the presence of these SNPs and measured concentrations of pro-inflammatory cytokines were further analyzed for association with persistent fungemia (⩾5 days of positive blood cultures) in 325 Americans with candidaemia. Other variables were assessed including type of *Candida* spp. identified, total parenteral nutrition (TPN), dialysis dependence, malignancy, immunocompromised state, renal/liver failure. Variables with $p < 0.10$ on univariate analysis were further analyzed using multivariable logistic regression.
Results: None of the SNPs examined were associated with susceptibility to candidaemia. Mean age of candidaemia patients was 55 years (±21.3), with 44% female, 32% African American, 58% immunocompromised, 31% active malignancy, 10% neutropenic, 43% recent surgery, 21% receiving total parenteral nutrition (TPN), 12% dialysis, 25% liver disease, and 49% ICU residence. The following species were identified: *C. albicans* (43%), *C. glabrata* (27%), *C. parapsilosis* (17%), *C. tropicalis* (12%), *C. krusei* (3%), >1 *Candida* species (6%). Overall, persistent fungemia occurred in 15% of cases. In the multivariable model, persistent candidaemia was significantly associated with (OR, [95% CI]): TPN (2.69 [1.28–5.62]), dialysis dependence (3.45 [1.36–8.74]), IL10 rs1800896 (3.07 [1.31–7.12]) and IL12B rs41292470 (4.34 [1.38–13.8]). In addition, significantly lower pro-inflammatory cytokine concentrations were measured in serum from patients with persistent fungemia.
Conclusions: SNPs in IL-10 and IL12B were associated with persistent fungemia in candidaemia patients, which also associated with low serum concentrations of pro-inflammatory cytokines. This may provide insights for future targeted management strategies for patients with this high mortality condition.

O410 An echinocandin vs. a comparator antifungal in *Candida* bloodstream infections: a meta-analysis

Y. Golan, D. Harrison, K. Fahrbach (Boston, US)*

Objective: Mortality from *Candida* bloodstream infections (CBSI) is high if not treated adequately. In recent clinical trials of CBSI, treatment with an echinocandin resulted in higher success rates. However, superiority has not been demonstrated. Given similarities in trial design and definitions, data from these trials can be combined in a metaanalysis. We compared the efficacy, measured as global clinical success at the end of intravenous therapy, of an echinocandin vs. a comparator AF agent in the treatment of CBSI.
Methods: We used standard metaanalysis methodology and a random effect model. We conducted a literature search to identify all randomized, controlled, trials (RCTs), assessing the efficacy of an echinocandin (caspofungin, micafungin, anidulafungin) in CSBI. To evaluate whether the comparator (fluconazole or amphotericin preparations) success rates were consistent with previously published data, we conducted an additional literature search of all RCTs in which these agents were evaluated. Articles were reviewed independently by 2 reviewers. The corresponding authors and sponsors were approached for additional data.
Results: 8 RCTs were included: 3 comparing an echinocandin to comparator antifungal (CAF), 1 comparing two echinocandins, and 4 comparing amphotericin preparations to fluconazole. The summary odds ratio for global success was 1.58 (95% CI 1.07–3.54) in favor of echinocandin therapy. The removal of a high-recruiting center that had a high anidulafungin response-rate had an insignificant impact on the results (OR 0. 1.46 95% CI 1.10–1.94). Global success rates observed in all included RCTs of fluconazole (5 treatment arms), amphotericin (4 arms) and the echinocandins (5 arms) were 62%, 73%, and 83%, respectively. In the echinocandin trials, success rates observed in the CAF arms were consistent with prior published trials. In an additional analysis that included only CBSI caused by *C. albicans*, echinocandin therapy resulted in higher success rate vs the CAF (74%; 95% CI 69–78% vs 59%; 95% CI 51–66%).
Conclusions: In patients with CBSI, as compared to amphotericin preparations or fluconazole, echinocandin therapy is associated with higher global success rates. This better effect is maintained when restricting the analysis to *C. albicans*, suggesting that effectiveness differences may be unrelated to the level of fluconazole susceptibility.

O411 Candidaemia in neonatal intensive care unit, Malaysia: a success story

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Objectives: Neonates in intensive care units were susceptible of having blood stream infections because of invasive procedures and underdeveloped immune system. Prolonged hospitalization and broad spectrum antibiotics further risk the development of candidemia. This report was written to share the success story in controlling the candidemia after a major outbreak in 2004 by adopting strict infection control practice, in which indirectly resulted in the reduction of candidemia in our neonatal intensive care unit (NICU).
Methods: This is a retrospective laboratory based analysis study from January 2001 to August 2009. Spectrum and distribution of *Candida* isolated from blood cultures taken from the NICU at Hospital Universiti Sains Malaysia before and after a major outbreak in 2004 was analyzed. The prevalence of candidemia before and after outbreak was compared and the trends of *Candida* spp. were determined. *Candida* spp. was identified using standard microbiological tests and a commercialized identification system, API-32C system.
Results: Altogether, 413 *Candida* were isolated during the study period. Generally, there was a significant reduction in the prevalence of candidemia after 2004 for both *Candida albicans* and non-*albicans*. The reduction was sustained for the past five years. *Candida parapsilosis* (40%) was the predominant species isolated followed by *Candida albicans* (24.8%), *Candida glabrata* (15.2%) and *Candida tropicalis* (12.4%).
Conclusion: Adherence to strict infection control practice is important to control candidemia. *Candida parapsilosis* was the major species implicated in candidemia among our neonates.

O412 Is there any factor prompting early central venous catheters removal from cancer patients with candidaemia?

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Background: Few studies have addressed the timing of CVC removal as a prognostic factor of mortality in cancer patients with candidemia.
Objective: To evaluated the clinical factors associated with early central venous catheter removal in cancer patients with candidemia who survived >3 days after the index blood culture.
Methods: This is a retrospective analysis from a candidemia study conducted between January 2001 and June 2005 at an oncology cancer center in Brazil. Eligible patients were those whose catheters were eventually removed. We excluded patients who died within 72 h after the candidemia onset or had catheters in place for ⩽48 h. Early catheter removal was defined as those catheters withdrawn ⩽72 h after the onset of candidemia.
Results: We enrolled 164 patients with a 10.4% overall mortality rate. Multivariate analysis revealed the temporary nontunneled central venous catheter type (OR, 5.06; 95% CI 2.16–11.83) as the only variable independently associated with early removal. Among the 82 episodes not catheter related, 52 catheters (63%) were removed at doctor's discretion due to the urgency for cancer treatment continuance. Most of these removal ($n = 46$) occurred within 3 days of the index blood culture. No differences in the overall mortality rates were seen among patients with early or delayed catheters removal ($P > 0.05$). Nevertheless, stratified analysis showed a survival benefit ($P = 0.04$) of early removal in the subgroup of patients with performance Karnofsky score >60.

Conclusion: This study showed short-term nontunneled central venous catheter type as the only factor associated with early catheter removal. There were a high proportion of removed catheters with the purpose of not interrupting the underlying cancer treatment. Overall, early catheter removal did not have a beneficial impact on mortality. Nevertheless, the observed favorable survival benefit of early over late catheter removal in the subgroup of patients without significant medical illnesses merits further investigation. Clinical trials enrolling sufficient number of homogeneous patients are necessary to analyze the impact of central venous catheter management on the outcome of cancer patients with candidemia.

Strategies to stay one step ahead of bacteria (Symposium supported by Janssen-Cilag)

S413 The bacterial challenge: time to react

J. Garau (Barcelona, ES)*

The changing pattern of antimicrobial resistance presents a challenge which needs to be met on many fronts. This, combined with the lack of development of new antimicrobials, necessitates a review of current treatment strategies. Strategies which will be discussed in this symposium will include the benefits of appropriate empirical antimicrobial treatment for serious infections, and more rational ways of administration (higher doses and varying infusion times) of some antimicrobials. Resistance development is well monitored in Europe, with resources such as the European Antimicrobial Resistance Surveillance System (EARSS), and additional country and local surveillance (e.g. PEG-surveillance in Germany, Austria and Switzerland). Data will be presented on resistance profiles and evolution rates within the intensive care unit, where the challenge is to contain and prevent the spread of resistance. These data are critical for the rational choice of adequate empirical antibiotic treatment which will not lead to failure. This can be achieved using broad-spectrum, potent agents, which are least likely to select for resistance, before changing to a more targeted therapy (de-escalation) once susceptibilities have been determined. These methods allow us to use our limited armamentarium effectively. If this approach is not followed, the risk of failure and increased mortality associated with initial inadequate antibiotic treatment, the emergence of resistant strains while on therapy, and their subsequent spread to others, are the main consequences. The number of new antibiotics to treat multidrug-resistant pathogens in the pipeline are too few, particularly those against Gram-negative bacteria and those with new mechanisms of action. New strategies are needed to close the gap between the burden of infections due to multidrug-resistant bacteria and the development of new antibiotics.

S414 Optimizing β-lactams by reducing resistance development

D. Livermore (London, UK)*

The development of resistance to antibiotics has long been a thorn in the side of the infectious disease physician. Dr Livermore will discuss current resistance issues from a microbiological perspective. The concept of potency and how it relates to minimal inhibitory concentrations (MICs) and breakpoints will be covered, with particular reference to carbapenems and *Pseudomonas aeruginosa*, where simple mutations leading to loss of the 'carbapenem-specific' porin OprD can confer frank resistance to imipenem whereas resistance to meropenem or doripenem additionally requires some upregulation of efflux. For reasons that remain obscure, these latter combinations of mechanism usually have a lesser effect on doripenem MICs than those of meropenem. In these circumstances there is potential for the dosing of carbapenems to affect the likelihood of resistance development, though any practical significance remains to be confirmed in clinical studies, particularly for the meropenem / doripenem comparison.
In contrast to *P. aeruginosa*, carbapenem-resistance in *Acinetobacter* spp. is due to OXA-carbapenemases. Some low-level producers may remain marginally susceptible to imipenem (not meropenem or doripenem) but there seems little general scope to overcome resistance by dosage adjustment for any of the compounds. Among Enterobacteriaceae, carbapenem resistance can arise either via combinations of porin loss together with production of extended-spectrum or AmpC-lactamases or via the production of true carbapenemases. The former mechanism, mostly seen in *Klebsiella* or *Enterobacter* spp. affects ertapenem more than any other analogue and this compound therefore seems most likely to act as a selector; the true carbapenemases – including IMP, VIM and NDM (New Delhi Metallo) zinc-enzymes as well as the KPC and OXA-48 serine types affect all carbapenems, and it seems unlikely that any could be overcome by dosage adjustment, though some might, in the future, be overcome by carbapenem/inhibitor combinations.

S416 Optimizing β-lactam antibiotics by maximizing PK/PD

F. Pea (Udine, IT)*

Consideration of pharmacokinetic (PK) and pharmacodynamic (PD) principles can be useful to optimise dosing of β-lactam antibiotics. β-lactams have time-dependent action, with time above the minimal inhibitory concentration (MIC) critical for maximum efficacy. PK/PD data can be used in Monte Carlo simulations to predict probabilities of obtaining an exposure target for a given dose. Evidence from Monte Carlo simulations, animal models and clinical studies have led to real-world recommendations, such as using extended infusions and other strategies to maximise efficiency of β-lactams. Prolonged and continuous infusions can give better efficacy, can lower the likelihood of resistance developing through higher trough levels and eliminate or at least reduce the need to increase the dose. β-lactam antibiotics that can be dosed via continuous/prolonged infusions include penicillins, cephalosporins and carbapenems. However, data should be assessed for each antimicrobial. For example, doripenem exhibits linear pharmacokinetics and does not accumulate with repeated dosing over 7 days, which supports its use as a prolonged infusion. Dr Pea will compare differences between carbapenems and classic β-lactams. Dosing strategies for different carbapenems will be discussed, including their stability and safety profiles. Practical considerations for prolonged infusions include having knowledge of the relationship of serum levels to target organ levels for different antimicrobials and an awareness of differences in PK and PD parameters among diverse patient groups. Dr Pea will conclude with a discussion on which particular patient populations, such as those with *Pseudomonas* infection, may benefit most from continuous infusions. He will also discuss the effect of different comorbidities, such as use in patients with severe sepsis or septic shock and with renal failure or glomerular hyperfiltration, and how their pathophysiology can affect drug levels.

S417 Novel β-lactams: what makes them different?

T. Welte (Hanover, DE)*

Dr Welte will discuss β-lactams which are newly approved and in late-stage development from a clinical perspective. Discussion will include summaries of *in vivo* data including pivotal clinical studies, recent meta-analysis, such as activity studies against *Pseudomonas* and *Acinetobacter*, and new clinical data on carbapenems, such as doripenem data dosed at 1 g. The use of intravenous antibiotics for patients with renal failure will be discussed, including considerations when using hemofiltration and continuous renal replacement therapy (CRRT). Comparative safety data will be presented for the carbapenems and recent health economic and hospital utilization datasets will also be discussed. Dr Welte will illustrate his talk with examples, particularly of patients who have failed therapy, with reasons for failure, which can be complex. Techniques and approaches to minimise the possibility of treatment failure will be discussed, such as appropriate initial empiric treatment and prolonged infusion. The presentation will conclude with a brief review of β-lactams currently in the pipeline including ceftobiprole, ceftaroline, and faropenem.

Cytomegalovirus infection: strategies, recommendations and future solutions (Symposium supported by DiaSorin)

S422 Epidemiology of human cytomegalovirus with focus on mother/child transmission

G. Jahn (Tubingen, DE)*

Human cytomegalovirus (HCMV) affects 30–100% of adults worldwide depending on geography and lifestyle by the age of 40 years. Two possible sources of sexual transmission of HCMV are virus in the female cervix and in semen of men. The frequency of HCMV infection in adults correlates with the number of sexual partners and sexual experience. This β-herpesvirus is the virus most commonly transmitted to the fetus before birth, occurring in 0.3–2% of all live births. Another even much more frequent transmission route of mother-child is breastfeeding. The local viral reactivation of the HCMV antibody positive mothers during lactation is highly frequent and self limited and follows unimodal kinetics. The vertical transmission rate from mother to child is about 40%. Also horizontal HCMV transmission from newborns and young children to their mothers or other individuals taken care of those infants happens. Children may shed the virus in saliva and urine for months or years, but the relative portion of this kind of horizontal transmission from children to children or from children to adults is unknown. Also the horizontal transmission among adults, besides sexual intercourse, is not well descript, but unlikely. In medical care, HCMV can be transmitted by blood products or organ transplantation. As with all other herpesviruses, HCMV has the ability to establish latent/silent infection in the host after primary infection. Activation from silent infection can occur under immunosuppression, during pregnancy and during lactation. Individuals can be infected with multiple strains, meaning secondary infection as exogenous reinfection or endogenous reactivation may occur. HCMV disease with various symptoms can result from either primary or secondary infection, also in primary infection disease is more severe and more frequent. The outcome of congenital HCMV infection is variable. About 10–15% of infected newborns exhibit clinical symptoms at birth such as CNS sequelae with hearing loss, cognitive impairment and cerebral palsy. Remarkably from 85–90% of HCMV infected newborns who are asymptomatic at birth will later develop hearing loss in about 15%. In both symptomatic and clinical asymptomatic newborns, sequelae often are not apparent in the first months or years of life. That all is an urgent need for specific HCMV diagnostic procedures and interpretations for pregnant women and newborns to guide the solid management of fetal/infant infection.

Contemporary challenges in β-lactamase inhibition

S435 Inhibition of KPC β-lactamase

J. Spencer (Bristol, UK)*

The growth of antimicrobial resistance, and in particular the proliferation of ESBL-producing Enterobacteriaceae, means that carbapenems are increasingly front line therapy for nosocomial infections. In consequence numerous carbapenem-hydrolysing β-lactamases have emerged and begun to disseminate across geographic and species boundaries. Several class A carbapenemases have now been described. Of these the KPC enzymes are of the most immediate clinical relevance. Growing numbers of reports indicate the presence of KPC variants in Enterobacteriaceae, notably from the Eastern United States and Israel but also in China and a number of South American and European countries. KPC-expressing isolates frequently contain additional β-lactamases as well as other resistance determinants, severely limiting treatment options and affecting patient outcomes.

Although class A carbapenemases have been extensively studied, the molecular basis for this activity has remained obscure in light of their close structural resemblance to other class A enzymes. It is now established that the acylenzyme formed when carbapenems react with class A β-lactamases such as TEM or SHV can adopt two conformations, and that associated tautomerisation ultimately results in inhibition. However, equivalent studies are yet to be reported for the species formed when carbapenems associate with class A carbapenemases. Using X-ray crystallography we have now studied the interactions of the carbapenem meropenem with a model class A carbapenemase (SFC-1 from *Serratia fonticola*) in which we have used directed mutagenesis to trap the substrate and acylenzyme complexes. These structures show that SFC-1 binds carbapenems in a single well defined orientation where, in contrast to carbapenem-inhibited enzymes like SHV, the β-lactam amide nitrogen remains close to the active site Ser-130. We propose that this model represents productive binding of substrate and is likely to apply to other class A carbapenemases, including KPC enzymes.

One approach to combatting carbapenemase-producing organisms is to combine β-lactams with an appropriate β-lactamase inhibitor. However, available β-lactamase-inhibitor combinations are variably effective against carbapenemase producers. Accordingly, a number of β-lactamase inhibitors under development have been tested against KPC and other class A carbapenemases. Recent results in this area will be reviewed.

S437 Designing class C inhibitors

M. Page (Basel, CH)*

Many Gram-negative bacteria, especially *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Enterobacteriaceae, including *Enterobacter*, *Citrobacter* and *Serratia* species, possess chromosomally encoded Class C β-lactamases (AmpC) that are important determinants of resistance towards a broad range of β-lactam antibiotics. Even carbapenems lose activity when confronted with class C lactamases if the organism also has restricted outer membrane permeability. For example, loss of the OprD outer membrane porin of *P. aeruginosa*, which is essential for the rapid entry of imipenem into the periplasm, will lead to high-level resistance if a class C β-lactamase is expressed. In the last decades, a number of AmpC enzymes have been mobilized on plasmids or other genetic elements and have spread to organisms such as *Escherichia coli* and *Klebsiella pneumoniae* that did not usually express AmpC. Carbapenem-resistant strains of *K. pneumoniae* that have altered porin expression and a plasmid-encoded AmpC β-lactamase have been reported from several countries. In general, class C lactamases are not well inhibited by the clinically available β-lactamase inhibitors clavulanic acid, sulbactam and tazobactam. Insights into the mechanism of class C β-lactamases have aided the rational design of specific inhibitors for this group of enzymes that also have the potential to been optimized for penetration of the outer membrane of *P. aeruginosa*. This presentation will review the fundamental steps in the mechanism of class C β-lactamases and how this knowledge can be applied to the design of novel inhibitors.

S438 OXA carbapenemases and their inhibition

M. Galleni (Liège, BE)*

The catalytic efficiency of the class D β-lactamase depends critically on an unusual carboxylated lysine as the general base residue for both the enzyme acylation and deacylation steps of catalysis. Different class D β-lactamase's X-ray structures indicate that the active site Lys-70 is surrounded by a hydrophobic core comprising residues such as Val-117, Phe-120 and Trp-154. Evidence is presented that the interaction between the indole group of Trp154 and the carboxylated lysine is essential for the stability of the post-translationally modified Lys70. Substitution of Trp154 by Gly, Ala or Phe yielded non carboxylated enzymes which displayed poor catalytic efficiencies and reduced stability when compared to the wild type OXA-10. A comparison of the three dimensional structures of the different proteins also indicated that the Trp154 mutations did not modify the overall structures of OXA-10 but induced an increased flexibility of the omega-loop in the active site.

Finally, the deacylation impaired W154A mutant was used to determine the structure of the acyl-enzyme complex with benzylpenicillin. Stopped-flow and quenched-flow experiments indicate that the deacylation step is clearly rate limiting for the OXA-10 β-lactamase. The observation of acyl-enzyme complex with the K70C mutant by X-ray crystallography also suggests that the deacylation step is rate limiting but the acylation step is also affected for the lysine mutants. Indeed, the values of the acylation constants (k2) are clearly lower than with the wild-type enzyme. The catalytic efficencies of the V117T mutant decrease for all substrates tested. The structure of the V117T OXA-10 mutant indicates that the Lys-70 is partially carboxylated at pH 8.0 in monomer A whereas monomer B contains a non-carboxylated Lys-70. This confirms that the valine residue in the hydrophobic core is important to promote the carboxylation of the Lys-70.
It has been reported that class D β-lactamases are inhibited by chloride ions. Interestingly, in we could show that the inhibiton is due to the replacement of the side chain carboxylate group of the modified lysine by chloride ion.
Finally, the structural modifications induced also the apparition of a detectable carbapenemase activity in OXA variants.
Finally, the deep analysis of structure-function relationship for class D β-lactamases yielded a new approach for the selection and synthesis of new inhibitors.

Community-acquired MRSA: emerging cause of pneumonia

S441 Pathogenicity: what does question the role of PVL?

A. Norrby-Teglund (Stockholm, SE)*

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has emerged as a major health problem world-wide. CA-MRSA is a frequent cause of skin and soft-tissue infections, and has also been reported to cause rapidly progressing life-threatening infections with unusually severe pathology, including necrotizing pneumonia, severe sepsis and necrotizing fasciitis. A large number of epidemiological and clinical studies have established a strong association between these severe CA-MRSA infections and strains harbouring the pore-forming toxin Panton-Valentine Leukocidin (PVL). Although compelling, epidemiological data alone is insufficient to establish a role of PVL in disease pathogenesis. However, this issue has been addressed in a large number of studies utilizing different experimental systems including PVL-deficient mutants and various *in vivo* models. The results of these studies have been highly conflicting, and consequently, this topic is a matter of great debate in the staphylococcal field. The current progress toward understanding the enhanced virulence potential of CA-MRSA with special emphasis on PVL, but also including other relevant staphylococcal virulence factors, will be summarized and discussed during this talk.

S442 Epidemiology and epidemicity of CA-MRSA pneumonia

G. French (London, UK)*

Healthcare-associated MRSA (HA-MRSA) infection is now widespread throughout the world. Recently, new strains of community-associated MRSA (CA-MRSA) have emerged that affect people with no history of healthcare contact. These are community strains of MSSA that have acquired mecA. They are generally more virulent than HA-MRSA and can cause primary infection in healthy people, including children. CA-MRSA often possess genes encoding the putative virulence factor Panton-Valentine Leukocidin (PVL). CA-MRSA clones can be defined by multilocus sequence (ST) and SCCmec type. CA-MRSA have appeared in most countries of the world. They are common in the USA and much less common, but increasing, elsewhere. There are many different clones whose distribution differs geographically. Some are particularly successful. USA300 (ST8-IV PVL+) has replaced USA400 (ST1-IV PVL+) as the commonest type in the USA. The 'European clone' (ST80-IV PVL+) is common in Europe and the 'South West Pacific' clone (ST30-IV PVL+) in the Far East and South America. In Europe there is great clonal diversity and new types are continually being reported, including the pig-associated ST398 strain (usually PVL–) that is common in Denmark and the Netherlands but now spreading elsewhere. Although new CA-MRSA clones continue to appear, successful older ones are being introduced into new geographical areas by travellers and immigrants. CA-MRSA pneumonia is uncommon worldwide but increasing. It may follow influenza in previously healthy people and may cause fatal necrotizing disease, usually associated with PVL-producing CA-MRSA. PVL-positive staphylococci produce necrotizing pneumonia in animal experiments but the role of PVL in human disease is still under debate. Although PVL+ USA300 is the most frequent strain causing pneumonia, this may be because it is common; it remains to be seen whether other types of CA-MRSA, including PVL– strains, also have this ability. With the continuing spread of CA-MRSA and the on-going influenza pandemic, we can expect the incidence of severe CA-MRSA pneumonia to increase. HA-MRSA pneumonia in ventilated patients is well recognised and has a poor prognosis. CA-MRSA is now spreading in hospitals and becoming increasingly multi-drug resistant (MDR). Physicians should be aware of the threat of hospital-acquired pneumonia with virulent, PVL-producing MDR CA-MRSA and national and international organisations should provide appropriate surveillance systems for CA-MRSA.

Preventing catheter-related infection: light and shadow

S453 Evidence-based practice to reduce CVC-related infections

W. Zingg (Geneva, CH)*

Many patients require a central venous catheter or an arterial line. Although indispensable and of benefit, vascular access devices pose a potential risk of complications due to central line-associated bloodstream infections (CLABSI). The risk for CLABSI varies between 1 to 7 episodes or more per 1000 catheter-days depending on ward-type, institution and socio-economic status of the country.
Most successful CLABSI prevention strategies are multimodal and include a combination of effective single intervention measures. Some measures are "procedural", such as using maximal sterile barrier precautions at catheter insertion, avoiding the femoral site, strict hand hygiene and applying a non-touch technique for catheter handling, opening hubs on antiseptic-impregnated pads, change of tubing only when indicated, and avoiding unnecessary access to the system. Furthermore, any indication for a central line insertion must be justified and catheters should be removed as soon as possible. Other intervention measures are more "technical", such as chlorhexidine-containing products for skin antisepsis, chlorhexidine dressings at the catheter insertion site, impregnated catheters with antibiotics or chlorhexidine and silver-sulfadiazine, access devices coated with silver particles, and closed infusion systems. A promising approach in CLABSI prevention is the use of lock-solutions consisting of taurolidine-citrate, ethanol, EDTA or citrate/methylene-blue/paraben.
Although there is evidence that some intervention combinations or bundles are effective, no specific combination should be considered superior to another. However, effort, complexity and costs may limit the possibility to apply all measures together. Even more important, infection prevention measures, and especially procedures, are of no use if they are not respected in daily practice. Most episodes of CLABSI are identified in the intensive care unit where care is complex. In such an environment, attention may be drawn away from infection control measures. Thus, infection control measures must be simple and easy to integrate into daily practice. Furthermore, it is indispensable to promote an infection control culture among nurses and physicians to ensure that prevention measures are observed under stress conditions. Although technical devices may be of help in stress situations, the failure to adopt a safety culture cannot be replaced by the use of impregnated catheters, chlorhexidine dressings or lock solutions.

S454 Antimicrobials against biofilm-based catheter-related infections: new perspectives

G. Donelli (Rome, IT)*

Antimicrobials against biofilm-based catheter-related infections: new perspectives. In the last decades, different strategies have been developed to prevent microbial colonization of intravascular catheters through surface adsorption or incorporation in the device polymer matrix of antibiotic/antifungal agents. However, the currently available medicated catheters have shown to inhibit microbial biofilm formation for relatively short periods, mainly due to the massive release of the loaded antimicrobial agents in the first 24 h followed by a slow release at sub-inhibitory concentrations until drug exhaustion, this phenomenon involving the risk of emergence of antibiotic-resistant strains. To overcome these limitations, we focused our research efforts in developing different experimental approaches to prevent microbial colonization of central venous catheters based on the adsorption of antimicrobial agents to synthesized and properly functionalized polyurethanes with the aim to control drug adsorption and release. Firstly, we have impregnated appropriately functionalized polyurethanes with two antibiotics with different mechanisms of action, rifampicin and cefamandole nafate, along with pore forming agents such as albumin and polyethylene glycol. This *in vitro* model exhibited a good polymer/antibiotic affinity and the ability to obtain a controlled release of large amounts of antimicrobials for up to 23 days. These results suggest that the entrapping of antibiotic molecules and pore-formers in properly functionalized polyurethanes may represent a promising approach to prevent catheter colonization and onset of bacterial resistance. Other antibiofilm strategies we are dealing with concern: i) the development of antimicrobial polymers by the use of polyurethanes able to coordinate metal ions (Ag+, Zn2+, etc); ii) the exploiting of the biofilm matrix-degrading enzyme, Dispersin B, to allow a better penetration of antibiotics through the microbial biofilm, thus improving their activity; iii) the development of a magnetic nanoparticles-based targeting system to fight catheter-related infections by an in situ, on demand, antimicrobial treatment.

Fever in the returning traveller

S455 Fever in travellers from India and south-eastern Asia

N. Beeching (Liverpool, UK)*

This presentation will focus on the changing epidemiology of fevers imported from this region reflected in travel infection databases, and use brief vignettes to highlight important clinical diagnostic and management issues. As in all travellers, a detailed travel, exposure and immunisation history is essential, combined with knowledge of patient comorbidities that may predispose to acquiring certain infections. In general, malaria is less likely to be the cause of fever than other infections, contrasting strongly with the differential diagnosis in travellers arriving from Africa. However, recent increases in malaria transmission in tourist areas such as Goa remind us of the need to maintain a high index of suspicion and continually to review pretravel advice on prevention. The emergence of artemesinin resistance in the Thai-Myanmar border area is a concern for the future.

In most recently returned travellers, arbovirus infections such as dengue and chikungunya are common causes of fever and can be confirmed using molecular techniques in the first week of illness and serological tests thereafter. Importation of scrub typhus is less common than is tick typhus in travellers from Africa. Leptopirosis is associated with fresh water activities popular with tourists in South East Asia, such as white water rafting and "tubing", but imported schistosomiasis from this area is rare. Eosinophilia is more likely to be due to other enteric or systemic helminth or fluke infections.

Enteric fevers are common in travellers from the Indian subcontinent, with recent predominance of paratyphoid A, which is often resistant to fluoroquinolones and multidrug resistant to other first line agents. Empirical treatment while awaiting culture results and resistance data should avoid such agents and include a third generation cefalosporin and/or azithromycin. Antimicrobial resistance is common in infections imported from this area, including extended spectrum β-lactamase producing Gram negative bacteria, and multidrug resistant tuberculosis. Respiratory infections are common in any traveller, and influenza has been demonstrated to be the most frequent vaccine preventable infection acquired by tourists in South East Asia. Recent experience with SARS and ongoing concerns in the region about avian influenza remind us of the continued risk in this region of the emergence of novel virus infections spread by the respiratory route.

S456 Fever in travellers from South America

E. Gotuzzo (Lima, PE)*

In a study performed by Geo Sentinel and published recently, it was found that, among North Americans, fever after a travel to South America is not very common and that it includes malaria, salmonellosis, dengue, etc.

It is important to make geographic distinctions:

1. In Mexico: amebiasis, hepatic abscess and typhoid fever should always be included.
2. In Central America: typhoid fever and dengue are the main causes of fever. Histoplasmosis and toxoplasmosis have been detected in immunosuppressed patients.
3. In South America: Malaria by *P. falciparum* and *P. vivax* are frequent causes of fever. Since 1991, the frequency of typhoid has decreased with more than 90%. The strains of *S. typhi* continue to be sensitive to chloramphenicol, ampicilline and fluoroquinolones. By contrast with Asia, multidrug resistant strains have not been detected.

Dengue and enteric hepatitis are frequent causes of fever. In Peru, brucellosis (as well as in Mexico and Venezuela) and bartonellosis should be considered as important diseases.

Occasionally, regional mycoses such as histoplasmosis (exposure to caves) and paracoccidioidomycosis are seen.

S458 Computer-assisted diagnosis of fever in travellers

J. Van den Ende (Antwerp, BE)*

Kabisa, a computer based tutorial for tropical medicine, has been developed since 1992, the first version in foxpro, the second in C++, the third in MS Access XP, and the last in Delphi. (Van den Ende et al. 1997a) For developing countries it is a didactic tool that challenges the student with cases electronically assembled from a randomly generated disease and randomly chosen related presenting symptoms. The student should find the diagnosis with a set of disease characteristics, available in the context of a hospital in developing countries. The logical engine is based upon both pattern recognition and Bayesian logic. For a Western context the programme offers also an expert system for imported fever, based on a prospectively generated database of over 2000 patients.

The expert module is innovative with the suggestion of new tests based on the data already provided by the user. In this suggestion, a threshold driven logic is followed: (Pauker & Kassirer 1980) for the highest ranked diagnosis strong excluders are asked for, once this diagnosis is worked out less probable hypotheses are examined also. Ranking of suggested tests takes into account power, feasibility, risk and cost.

During the session, real examples will be played and the application of the logic shown. If wanted, detailed explanation of the programming can be given. Participants are invited to bring their laptop; the program will be installed and distributed for free.

Reference(s)

[1] Pauker, S. G. & Kassirer, J. P. 1980, "The threshold approach to clinical decision making", New England Journal of Medicine, vol. 302, no. 20, pp. 1109–1117.

[2] Van den Ende, J., Blot, K., Kestens, L., Van-Gompel, A., & Van-den-Enden, E. 1997a, "Kabisa: an interactive computer-assisted training program for tropical diseases", Med. Educ., vol. 31, no. 3, pp. 202–209.

Reducing hospital-acquired infections. What is new?

O459 Hospital resources and capabilities in dealing with highly infectious diseases: EuroNHID data from a survey of 44 isolation facilities in 14 European countries

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Objective: Highly Infectious Diseases (HIDs, e.g. Viral Haemorrhagic Fevers and SARS) are life-threatening, human-to-human transmissible diseases that may cause Public Health emergencies, requiring special procedures for their containment. To review isolation hospital resources, the European Network for Highly Infectious Diseases project conducted, through a specifically developed checklist, a survey in the facilities designed to deal with HIDs. These data from 44 facilities in 14 European Countries are described.
Methods: The checklist, including 10 items and 44 questions, was developed through a "networking strategy": a project partner with specific expertise sent drafts for comments and amendments. Final agreement had been reached during a meeting involving all partners. Facilities to be surveyed were selected by national authorities, and are those planned for giving care to patients affected by HIDs. In site surveys were conducted from March to November 2009.
Results: Totally, 481 hospital beds in 350 rooms are available, and in 185 of them it is possible to give intensive care. These facilities are mainly recently built/renovated (70% after 2000). Most of surveyed facilities use these beds routinely, while in 10 isolation facilities they are reserved to HIDs only. Among technical issues, about 90% of facilities are equipped with anterooms, negative pressure inside the isolation area, and high-efficiency filtration of exhausting air. Availability of other technical features (autoclave, sealing of the room, use of adequate material, communication systems, negative pressure indicators, self-closing doors) varies among countries. Four facilities have a BSL-4 laboratory in the same centre/city, while BSL-3 laboratories are available in the same centre/city for 36 facilities. In 60% of facilities, staff is specifically trained, including physicians and nurses with Infectious Diseases and Intensive Care background.
Conclusion: According to preliminary data analysis, generally the hospital resources in isolation are adequate, despite the fact that different levels of preparedness among different countries are still present, in particular in the field of intensive care capabilities and availability of trained staff. These data will represent a valuable tool both for surveyed facilities, in order to identify their strengths and weaknesses, and for European authorities, providing an "on-the-field" evaluation of European hospitals' capabilities in dealing with HIDs.

O460 Effects of selective digestive and selective oropharyngeal decontamination on bacteraemia and respiratory tract colonization with highly resistant micro-organisms

A.M. De Smet, J. Kluytmans, H. Blok, M. Bonten, M. Bootsma (Amsterdam, Breda, Utrecht, NL)*

Background: Selective Digestive tract Decontamination (SDD) and Selective Oropharyngeal Decontamination (SOD) were associated with improved day-28 survival in intensive care patients, but the effects on infections and respiratory tract colonization with Highly-Resistant Microorganisms (HRMO) are unknown.
Methods: SDD, SOD and standard care (SC), during periods of six months each, were evaluated in an open clustered group-randomized cross-over study in 13 ICUs, with the order of interventions randomized per center. SOD consisted of four times daily topical application of tobramycin, colistin and amphotericin B in the oropharynx. SDD consisted of SOD and topical application of the same antibiotics in the stomach and four days of intravenous cefotaxime. Cultures of respiratory tract were obtained twice weekly during SDD and SOD, and on clinical indication only during SC. HRMO were defined according to Dutch guidelines. All blood and respiratory tract culture results were evaluated.
Results: 5,927 patients were available for analysis: 1,989 (SC), 1,904 (SOD) and 2,034 (SDD). Compared to SC, odds ratios (OR) for ICU-acquired bacteremia were 0.48 (95% CI 0.38–0.60) during SDD 0.66 (95% CI 0.53–0.82) during SOD. OR for ICU-acquired bacteremia caused by HRMO during SDD were 0.41 (95% CI 0.18–0.94) as compared to SC, which corresponds to a rate reduction of 59%, an absolute risk reduction (ARR) of 0.6% and a number needed to treat (NNT) of 170. As compared to SOD, the OR for SDD was 0.37 (95% CI 0.16–0.85), which corresponds to a rate reduction of 63%, an ARR of 0.7% and a NNT of 145. ICU-acquired colonization of Gram negative bacteria was highest among patients receiving SC. ORs for acquiring HRMO colonization, as compared to SC, were 0.58 (0.43–0.78) and 0.65 (0.49–0.87) for SDD and SOD respectively, corresponding to 38% and 32% rate reductions, 5.5% and 4.6% ARR and with NNT of 18 and 22, respectively. Acquired colonization with cefotaxime-resistant or colistin-resistant pathogens was lowest during SDD.
Conclusions: As compared to SC, ICU-acquired bacteremia and respiratory tract colonization with HRMO were 48% and 59% lower during SDD and acquired respiratory tract colonization with HRMO was 38% lower during SOD.

O461 Prevention of catheter-related bacteraemia with a daily ethanol-lock in haematology patients with tunnelled catheters. Randomized placebo-controlled trial

B.J. Rijnders, L. Slobbe (Rotterdam, NL)*

Objective: Catheter-related bacteraemia (CRB) results in significant attributable morbidity and mortality. In this randomized double-blinded placebo-controlled trial, we study the efficacy and safety of a daily ethanol-lock on prevention of CRB in patients with a tunnelled central venous catheter (CVC).
Methods: From 2005 until 2008, each CVC lumen of adult haematology patients was locked for 15 minutes per day with either 70%-ethanol or placebo, following which the lock-solution was flushed through. As a primary endpoint, rates of endoluminal CRB in each group were compared.
Results: The catheter-based ITT-analysis was based on 376 patients, accounting for 448 catheter episodes and 27,745 catheter days. For ethanol-locks, the incidence of CRB per 1000 days of CVC-use was 0.70 compared to 1.19 in patients allocated to placebo (incidence rate ratio 0.59; $p = 0.19$). In patients who classified for endoluminal CRB according to the strictest definition (positive hub culture and concurrent bacterial strain detected in blood) a 3.6-fold, but not statistically significant reduction of CRB was observed for patients receiving ethanol (2/226 versus 7/222; $p = 0.103$). No life-threatening adverse events were observed. More patients receiving ethanol discontinued lock therapy (11/226 versus 1/222; $p = 0.006$) or continued with decreased lock frequency (10/226 versus 0/222; $p = 0.002$), due to subjective discomfort, especially facial flushing and drowsiness.
Conclusion: In this study, the use of ethanol-locks non-significantly reduced the incidence of endoluminal CRB. However, further studies are needed as the overall low incidence of endoluminal precludes definite conclusions. Alternative sources of bacteraemia, like microbial translocation during mucositis or exoluminal CRB may have been more important in this patient population.

O462 Different strokes: a co-relational modelling study of common (community and acute hospital) HCAI reduction targets, variable dynamics and antibiotic prescribing

I. Maitland, R. Kato, M. Ibeto, M. Qulaghassi, A. Guleri (Blackpool, UK)*

Objectives: Reducing healthcare associated infections (HCAI) in hospitals & community has been priority for department of health (DH). Blackpool Victoria Hospital, large district acute hospital covers

Blackpool PCT & North Lancashire PCT. DH conferred HCAI innovation technology award 2009 for most innovative use of technology (MRSA PCR) to significantly reduce MRSA. The comprehensive HCAI containment program (CHCP) including multiple interventions was introduced in BVH in 2008 with successful reductions: MRSA bacteraemia 78%, *C. difficile* infections (CDI) 45%; ESBL 25% and glycopeptide usage 32%. Antibiotic stewardship (AS) is a key area. Revised separate formularies for hospital and primary care (with restrictions on quinolones & 3rd gen cephalosporin) introduced. HCAI rates (CA & HA) are jointly submitted. We present a co-relational study from audit on GP antibiotic prescribing, comparative reductions in HCAI and new whole health economy (joint primary care–hospital) initiatives.

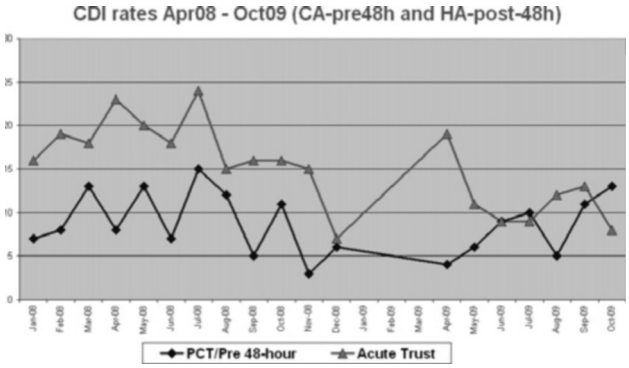
Methods: Data from audit (initial Aug08 & final Aug09) on antibiotic prescribing in a large GP surgery in North Lancashire [e.g. Compliance to formulary, choice/dose/duration/appropriate, etc); data on HCAI (pre48 h & post48 h) were analysed.

Results: The audit revealed 64.4% prescriptions had adequate indication; Recommended antibiotic was prescribed in 53.4% (321/601) & 70.9% (73/103) cases in the initial & final audit resp. The final audit revealed 37.9% (36/103) carried correct dose & duration of antibiotic while 61.2% (60/103) had only correct dose.

HCAI rates (Apr08–Sep09): CA-CDI reduced by 25% (Apr–Sep09 v/s Apr–Sep08) while HA-CDI reduced by 37%. CA-MRSA bacteraemia increased by 33.3% while HA-MRSA reduced by 50%. CDI-RCA across PCT & hospital (Jan–Oct09) reveal non-compliance with formulary, repeated & extended courses of antibiotics/susceptible (>65 yrs) population is the single predominant cause of 'avoidable/probably-avoidable' CDI.

Conclusions: HCAI travel freely between community & hospital. There is variation in the dynamics & resources of PCT & hospital ICTs. However, the key to a successful joint HCAI programme is team working & complimenting expertise, joint initiatives & real-time monitoring of the CHCP to align it to meet new challenges.

Data reflect only 37.9% of compliance to both accurate dose and duration. We recommend additional microbiologist & antibiotic pharmacist input, raising awareness about HCAI, infection control & antibiotic stewardship is required to improve GP prescribing.



O463 Decontamination of sink wastes and traps is associated with a reduction in Gram-negative sepsis in a neonatal intensive care unit

S. Knowles, M. Matheson, M. Walsh (Dublin, IE)*

Objectives: To compare the incidence of nosocomial Gram-negative sepsis in a neonatal intensive care unit (NICU) before and after the introduction of quarterly dismantling, cleaning and disinfection of sink wastes and traps.

Methods: Prospective surveillance of all late-onset (neonate >72 hours of age) nosocomial Gram-negative sepsis in the NICU over a 40 month period from July 2006 to October 2009. Gram negative bacteria isolated from blood or cerebrospinal fluid were included. Sepsis rates during 24 months before the introduction of quarterly cleaning and disinfection of sink wastes and traps in July 2008 were compared with the 16 months following. Patient demographic information collected includes sex, gestation, birth weight, multiple birth, day of onset of sepsis and outcome. Data from environment screening of sink faucets and drains are documented.

Results: Twenty one episodes of sepsis occurred in 21 neonates during the period from July 2006 to June 2008. There was 1 meningitis (*E. coli*), 1 meningitis and bacteraemia (*Enterobacter* species) and 19 bacteraemia's (6 *E. coli*, 4 *Klebsiella pneumoniae*, 7 *Enterobacter* species, 1 *Proteus mirabilis* and 1 *Pseudomonas aeruginosa*). After introduction of quarterly cleaning and disinfection of sink wastes and traps 3 episodes of sepsis have occurred in 3 neonates during the 16 months from July 2008 to October 2009. These were 3 bacteraemia's (2 *Klebsiella* species and 1 *Acinetobacter* species). Before introduction of this practice, there were an average of 10.5 episodes of Gram-negative sepsis per annum compared to 2.25 per annum currently ($p < 0.01$).

Conclusions: No significant infrastructural work took place during this 40 month period. There was no significant change in medical, nursing, hygiene or infection control staffing numbers. The antibiotic policy remained unchanged. The number of admissions to the NICU was stable (1239 in 2006; 1215 in 2007 and 1232 in 2008), although the percentage of neonates <1500g birth weight increased from 99 (8%) in 2006 to 137 (11.3%) in 2007 and 154 (12.5%) in 2008. The reduction in the rate of Gram-negative sepsis as very low birth weight admissions increased was unexpected. Methods to reduce Gram-negative sepsis are complex and multifactorial infection control measures are required. Nonetheless, we found quarterly dismantling, cleaning and disinfection of sink wastes and traps was associated with a reduction in Gram-negative sepsis.

O464 Hand hygiene compliance in 13 European intensive care units

L. Derde, C. Brun-Buisson, M. Bonten on behalf of the MOSAR research consortium*

Objective: To quantify hand hygiene compliance (HHC) and its determinants in 13 European intensive care units (ICUs).

Methods: Between May 2008 and September 2009 15.223 hand hygiene opportunities were observed in ICUs in France (n = 3), Greece (n = 2), Italy (n = 1), Latvia (n = 1), Luxemburg (n = 1), Portugal (n = 2), Slovenia (n = 2) and Spain (n = 1). Observations were performed by trained research nurses at random dates, times and bed spaces during 6 months using a standardized protocol derived from the WHO "5 moments" method. No feedback of results or training of staff was allowed. A standardized test was used to assess inter-observer variation.

Results: The mean bed occupancy rate was 85% (58–99%) and ICUs had on average 13.11 (9–21) beds, with 61% of patients being ventilated (43–81%). The average nurse-to-patient ratio (per occupied bed) was 0.52 (0.34–0.91). HHC rates varied from 7% to 88% (mean: 48%). Nurses' HHC was higher than physicians' HHC (mean: 51 vs 39%) in all but two centers. Auxiliary nurses and other healthcare workers had intermediate HHC (42% and 44%, respectively).

When separated per indication category (i.e. hand hygiene before and after actions that require hand hygiene), HHC was higher in the latter group (43 vs 57%, $p = 0.003$).

45% of missed hand hygiene opportunities involved the use of gloves, mainly before performing an aseptic task or after possible body fluid contact. Gloves were used in 47% of opportunities missed by nurses compared to 30% for physicians ($p = 0.005$). No relationship between HHC and nurse staffing hours (correlation 0.049) or bed occupancy rate (correlation −0.314) could be demonstrated. In a standardized written test-scenario of 41 items, inter-observer variation was low, with a mean HHC scored of 31% (SD 7.18) compared to 29% in the referent test ($p = 0.250$). Preliminary data of 10 of the ICUs suggest that a hand hygiene improvement program (HHIP; WHO method) improves HHC significantly.

Conclusions: In these 13 ICUs, HHC varied from 7% to 88%. In general nurses had higher HHC rates than physicians. No correlation between nurse staffing hours or bed occupancy and HHC could be demonstrated. Preliminary data suggest that HHC was improved with a HHIP program (WHO method).

O465 A survey examining promotion of hand hygiene in healthcare through campaigns and programmes coordinated at a national/sub-national level

E. Mathai, B. Allegranzi, C. Kilpatrick, S. Bagheri Nejad, W. Graafmans, D. Pittet (Geneva, CH)*

Objective: The WHO First Global Patient Safety Challenge "Clean Care is Safer Care" (CCiSC) recognises the importance of nationally coordinated activities in achieving its goal of reducing healthcare-associated infection (HAI) through improved hand hygiene in healthcare. A baseline survey of existing hand hygiene national/sub-national initiatives was conducted in 2007 and repeated in early 2009 to assess current status and to generate information on factors contributing to their success.
Methods: Campaigns and programmes promoting hand hygiene in healthcare were identified through WHO regional offices and experts in the field. An online survey using a structured questionnaire was conducted during March-April 2009.
Results: In 2009, 38/38 campaigns/programmes identified (18/20 in 2007) completed the survey. Of the 38, 29 were active national/sub-national level initiatives from all WHO regions, except for Africa; 21 (72.4%) were initiated after the launch of CCiSC in October 2005. The main targets of hand hygiene promotion were general, district and university hospitals, with increasing coverage of long-term care facilities and primary care. The scope varied from awareness raising to well scaled-up activities with ongoing evaluation. Most activities (20/29) obtained funding from multiple sources with governments among the main funders; governments were responsible for initiating 25/29 (86.2%) campaigns/programmes. Through the 2009 survey, the facilitator role played by CCiSC in initiating activities and the support with tools and recommendations emerged very clearly. Barriers were identified, but the perceived significance of specific barriers varied considerably. Those related to commitment (priority and support) and resource availability were important across all regions. A range of indicators to measure the impact of the initiative was reported with process indicators being more common.
Conclusion: Hand hygiene is being promoted in healthcare in several countries at national/sub-national level with clear objectives, strategies and governmental support. Such embedding through policies and resource allocation is important for sustainability. Actions to improve commitment from different stakeholders are needed. Indicators for measuring impact need to be more uniform and more widely implemented. Further actions to initiate coordinated activities across the world, including countries with limited resources, are required.

O466 Effect of a multifaceted intervention on adherence to hand hygiene among healthcare workers: a cluster randomized trial

D. Mertz, N. Dafoe, S. Walter, K. Brazil, M. Loeb (Hamilton, CA)*

Objectives: Compliance with hand hygiene among health care workers (HCWs) is widely felt to be a key factor in reducing the spread of nosocomial infections. The objective of this study was to evaluate the impact of a multi-faceted intervention to increase adherence of hand hygiene among healthcare workers (HCWs) and assess the effect on the incidence of nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA).
Methods: A cluster randomised controlled trial was conducted from June 2007 to May 2008 in 30 units of three tertiary care hospitals in Hamilton, Ontario, Canada. Study units included eight medical, four general surgery and eight intensive care units. Following a three month baseline period of data collection, 15 units were randomly assigned to the intervention arm (performance feedback, small group teaching seminars, posters) and 15 to usual practice. Hand hygiene was observed during randomly selected fifteen-minute periods on each unit and the incidence of MRSA measured using surveillance specimens.
Results: 3812 (48.2%) of 7901 opportunities for hand hygiene in the intervention group resulted in adherence compared to 3205 (42.6%) of 7526 opportunities in the control group ($P < 0.001$). However, there was a significant increase of adherence compared to baseline adherence (15.4% in the control and 16.2% in the intervention group) for both groups (Figure). There was no reduction in the incidence of nosocomial MRSA in the intervention group.
Conclusion: Among HCWs in Ontario tertiary care hospitals, adherence to hand hygiene significantly increased with a multi-faceted intervention. Moreover, there was a marked increase in adherence to hand hygiene in both study groups. No resulting difference in MRSA rates between patients in the two study groups could be observed.

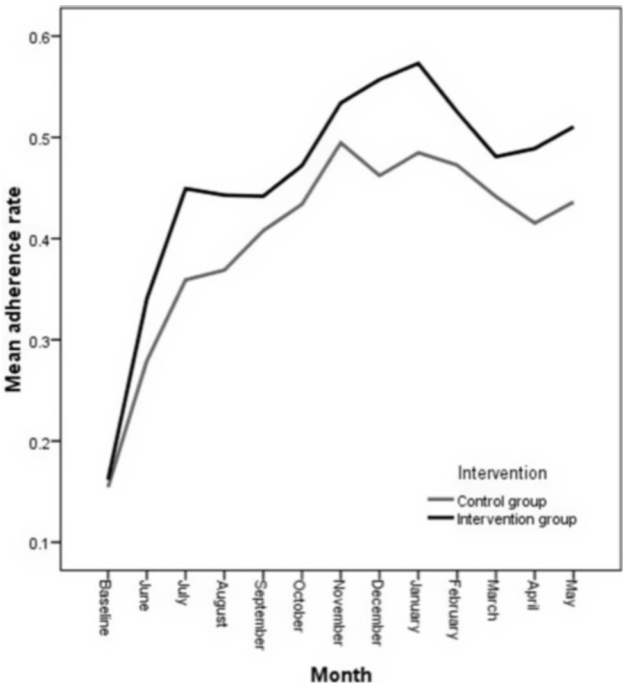


Figure. Mean adherence rates to hand hygiene in the control and intervention group.

O467 Glove use in infection control – is this a significant barrier to hand hygiene compliance?

C. Fuller, J. Savage, S. Besser, S.P. Stone (London, UK)*

Objectives: WHO guidelines stipulate that gloves are required for specific clinical procedures, but warn that they are not a substitute for disinfecting or cleaning hands. It has been suggested previously that wearing gloves could be a barrier to good hand hygiene compliance but the literature is divided as to whether healthcare workers (HCWs) are less likely to clean their hands when wearing gloves. We carried out a large multicentre study to determine whether wearing gloves was associated with poorer hand hygiene compliance.
Methods: 232 hours of observations (7020 observations) were carried out on 15 Intensive Therapy Units (ITUs) and 41 Acute Care of the Elderly/General Medical (ACE/GM) wards in 20 hospitals in England & Wales, whilst conducting a randomised controlled trial of an intervention to improve hand hygiene compliance (FIT trial N0256159318, NRR website). Glove use was not part of the intervention. Hand hygiene moments & behaviours were recorded using a rigorously standardised hand hygiene observation tool (the HHOT), and noting whether the HCW was using gloves or not. Compliance with & without gloves was compared overall & for different hand hygiene moments.
Results: Of the 7020 observations, 1729 (25%) were associated with glove use in both ITUs and ACE/GM wards. Gloves were used in 78% of high risk hand hygiene moments (aseptic technique, body fluid exposure) and in 16% of low risk moments (before & after patient

contact, after environmental contact). Overall hand hygiene compliance was lower when wearing gloves (42.6%) than when not wearing gloves (51.2%). The odds of HCWs cleaning their hands were less when gloves were worn (OR = 0.71; [CI 95% 0.61–0.82]). This was true for all hand hygiene moments. On ACE/GM wards there was a greater difference in overall compliance (35.3% with gloves v 49.5% without; OR = 0.56 [CI 95% 0.45, 0.69]) than on ITUs (50.1% v 54.9%).

Conclusions: This large study found that glove use was associated with worse hand hygiene compliance, and may present a barrier to HH. Modelling studies suggest that small increments in compliance may result in large reductions in transmission of organisms. It is therefore, possible that interventions to reduce unnecessary glove use & ensure appropriate compliance before & after glove use could have a significant effect on spread of infection. Further work examining the predictors of glove usage & associated hand hygiene by HCWs may be warranted to guide such interventions.

O468 Achieving sustained improvement in hospital hygienic cleaning using peer-group benchmarking

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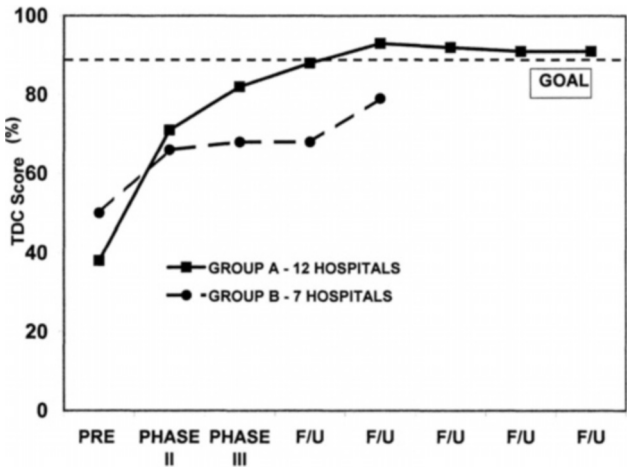
Objectives: Ongoing contamination of surfaces in the "patient zone" (Pittet 2007) has become increasingly recognized as playing a role in the transmission of major healthcare-associated pathogens. Over the past five years an indirect monitoring system has been used to improve environmental hygiene related to discharge cleaning in almost 100 hospitals. In view of these results, we undertook a prospective evaluation of a multi-hospital benchmarking program to further analyze this approach for improving the thoroughness of disinfection cleaning (TDC) at discharge.

Methods: The TDC of fourteen high touch objects was evaluated using a fluorescent dye based targeting method in two hospital systems consisting of 12 hospitals (Group A) and 7 hospitals (Group B) administratively related within each group but geographically and functionally independent institutions. A three phase intervention was utilized as previously described (ICHE 2008; 29: 1035–41). TDC scores were provided to the environmental services administration at each hospital and on a regular basis as part of system-wide quality assurance reviews for each system. At each meeting TDC scores were reviewed and the most effective programmatic and educational interventions shared.



Results: As noted in the Figure, pre-intervention (Phase I) TDC scores averaged 39% (95% CI 27.4 to 50.1) in group A hospitals and 53% (95% CI 33.1 to 72.8) in group B hospitals. Following education alone (Phase II), scores in both hospital groups improved with group A hospitals improving to 70.1% and group B hospitals to 68%. Subsequently (Phase III) and as a result of serial feedback and peer group benchmarking (F/Us), overall TDC continued to improve in group A hospitals to 88% and group B hospitals to 77%. As noted in theFigure, high levels of improvement were documented for sustained periods of time (to date, 6 to 16 months).

Conclusions: (1) Phase I of the study disclosed previously unsuspected differences in TDC despite the existence of similar cleaning policies in all hospitals. (2) Group benchmarking of TDC scores favorably impacted additional improvement in cleaning. (3) The ongoing transparency engendered by the system-wide programs has made it possible to sustain gains for up to 18 months. (4) The development of a patient-safety oriented, non-punitive environment as well as individual hospital and system-wide leadership support were recognized as critical components of the success of the program.

Antimicrobial consumption – experience from ESAC and other surveillance studies

O469 The ESAC point prevalence survey: antimicrobial prescribing in 2 age groups of elderly patients from 49 hospitals in 28 European countries in 2008

B. Amadeo, P. Zarb, G. Gavazzi, A. Muller, V. Vankerckhoven, P. Davey, H. Goossens on behalf of the ESAC Hospital Care sub-project Group*

Objectives: As ageing population raises fast, elderly aged above 65 years are usually considered as a one group in literature. However, if infections are more frequent and more severe in the elderly, they also have specific features related to different subgroups of the elderly patients. This study aimed to identify and to assess the variability of antimicrobial (AM) use between 2 age groups of elderly patients.

Methods: Data were extracted from 49 hospitals of the European Surveillance of Antimicrobial Consumption (ESAC) Point Prevalence Survey carried out during a maximum of 2 weeks from May to June in 28 European countries in 2008. The survey included all inpatients wards and collected information on the treated patients with indications and diagnoses. The analyses were restricted to patients above 65 years which were divided into 2 age year groups (G1: [65–75[; G2: $\geqslant$75).

Results: Data for treated patients were obtained for 1,579 patients in G1 and 2,132 patients in G2. Among all the treated patients, G2 received less AM combination (G1: 33%; G2: 24%) and parenteral AM (G1: 65%; G2: 57%). The top three AM classes prescribed were similar in both groups and corresponded to combinations of penicillins with β-lactamase inhibitors (G1: 18%; G2: 24%), fluoroquinolones (G1: 12%; G2: 14%) and third-generation cephalosporins (6% for both groups). Infections represented 74% and 83% of all indications in G1 and G2, respectively. Respiratory tract infections were the commonest infections in both groups (G1: 29%; G2: 33%) followed by skin-soft-tissues-and-bone-joint infections in G1 (18%) and urinary tract infections in G2 (23%). The proportion of surgical prophylaxis was lower in the oldest patients (G1: 69%; G2: 66%). The >1 day duration of surgical prophylaxis was 54% in both groups. The prescribed doses for 5 parenteral AM, i.e. benzylpenicillin, gentamicin, cefuroxime, piperacillin–tazobactam, and vancomycin were higher in G1 whilst amoxycillin and cefazolin were higher in G2.

Conclusion: The results of this study showed differences between the 2 elderly age groups, particularly in the proportion of AM combination, parenteral use, and infection site. It became clear that in line with the improved quality of life of the elderly population in industrialized countries, the treatment of G1 is more comparable to that of younger adults. Importantly, future analyses on AM use should take several age groups of the elderly population into consideration.

O470 The European Surveillance of Antimicrobial Consumption: point prevalence survey of antimicrobial prescriptions in 270 European nursing homes

B. Jans, K. Latour, E. Broex, A. Muller, V. Vankerckhoven, R. Stroobants, H. Goossens on behalf of the European Surveillance of Antimicrobial Consumption (ESAC) Nursing Homes subproject group*

Objectives: Facing the treat of antimicrobial resistance in healthcare settings, optimising the use of antibiotics (AB) in the nursing home (NH)

population is an important priority of quality of care. However, data on AB-use in European (EU) NHs are scarce. The European Surveillance of Antimicrobial Consumption (ESAC) NH sub-project team, funded by the European Centre for Disease Prevention and Control, carried out a methodology in order to measure and describe AB prescriptions among residents living in EU NH.

Methods: In April 2009, a PPS was carried out in 301 NH in 19 EU countries. Inclusion criteria for residents were to be present in the NH for at least 24 hours and to receive systemic AB on the day of the PPS. Data were obtained from nursing notes, medication administration records and staff in relation to AB prescribing, characteristics, risk factors and determinants at NH- and at resident level.

Results: Data were available for 17 countries and 270 NH (29.360 NH-beds). The mean number of beds by NH was 108 (20–621 beds). Among 27.614 eligible residents, 1740 (median 5.9%, 0–30%) received an AB on the PPS-day. In 20 NH no residents received AB. In the total NH-population 4% (0–57%) had an urinary catheter, and of these, 17% received an AB. Wounds were present in 10% of the population (0–75%) and AB were prescribed in 15% of them. Vascular catheters were uncommon (0.78%) but 36% of this sub-population used an AB. Among residents with AB, 24% had a recent hospital stay. In total 1757 AB molecules were used. AB were administered orally in 90%, parenteral in 9% and nasal (decolonisation MRSA) in 1%. 53% of all treatments concerned urinary tract (prophylactic: 55%) and 29% the respiratory tract (empirical: 92%). 51% of all prescribed regimens were empirical treatments (RTI: 53%, UTI: 23%), 32% was prophylactic (UTI: 89%, RTI: 5%) and 16% was for a documented infection (UTI: 72%). The prevalence of AB use was significantly lower in NH with regular training of prescribers ($p = 0.02$), with written guidelines for appropriate AB-use ($p = 0.01$) or with a NH therapeutic formulary ($p = 0.0002$) compared to NH without these tools.

Conclusion: Strong differences in AB-prevalence and device-use were observed in EU NH. Both micro (case-mix)- and macro determinants (cultural differences) partially contribute to these differences. The high proportion of AB-use for urinary tract, especially the important part of uroprophylaxis, was surprising and needs to be explored.

O471 Impact of medical care and coordination on antibiotic policy and consumption: data of the European Surveillance of Antimicrobial Consumption (ESAC) nursing home subproject

K. Latour, E. Broex, A. Muller, N. Drapier, V. Vankerckhoven, R. Stroobants, H. Goossens, B. Jans on behalf of the European Surveillance of Antimicrobial Consumption (ESAC) Nursing Home subproject group*

Objectives: The aim was to explore medical care and coordination in European nursing homes (NH) and their effect on antibiotic (AB) policy and use.

Methods: The European Surveillance of Antimicrobial Consumption (ESAC) NH subproject explored the medical care and coordination and AB policies by using a standardised questionnaire which had to be completed by participating European NHs.

Results: The questionnaire was completed by 270 NHs in 16 European countries. Medical care was provided by personal general practitioners (GP), by an employed medical staff or by both in 67.3%, 20.3% and 12.4% of the NH, respectively ($n = 266$). A NH working with GPs was visited by a median of 26.3 personal GPs per 100 NH beds (min.0.3-max.96.6 per 100 beds) while in other NHs the medical staff consisted of a median of 2 physicians (min.1-max.14). A coordinating physician (CP) of medical care was assigned in 68.4% of the NHs ($n = 256$). The most reported tasks of the CPs were to develop an infection prevention policy (77.7%), to train nursing staff (76.0%), and to develop medical care strategies (70.9%). The presence of a CP in a NH did not result in a significant lower number of AB prescriptions compared to NHs without a CP (median AB prevalence 5.78% vs. 5.71%; $p = 0.46$). However, NHs where the CP developed an infection prevention policy showed a significant lower rate of AB use in comparison to NHs where the CP did not have this specific task (median 5.2% vs. 8.7%; $p = 0.0097$). Furthermore, the presence of a CP led to a significant higher availability of a restrictive AB list compared to NHs without an assigned CP (median 17.4% vs. 6.3%; $p = 0.018$).

Private institutions were more likely to work with GPs than public NHs (59.2% vs. 40.8%), which in their turn had a greater tendency (84.9% vs.15.1%) to work with an employed medical staff ($p < 0.001$). Working with visiting GPs in the NH made it more difficult to develop a restrictive AB list compared to NHs with a medical staff (5.6% vs. 43.1%; $p < 0.001$). However, the number of visiting GPs per 100 beds did not significantly influence (categorical variable: <20, 20–40, >40 GPs/100 beds; median 5.06%, 6.19% and 6.67%, respectively) the prevalence of AB use ($p = 0.15$).

Conclusion: Although an impact of an appointed CP in the NH on the prevalence of AB use could not be demonstrated, his role in developing a restrictive AB list and infection prevention policy was clearly shown in our survey.

O472 The European Surveillance of Antimicrobial Consumption (ESAC) survey of wound prevalence and antibiotic use in 270 European nursing homes in 2009

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Objectives: To define wound prevalence, its determinants and its relation with antibiotic (AB) use in European nursing homes (NHs).

Methods: A point prevalence study, on AB use, characteristics of residents and characteristics of the NH was conducted in European NHs in 2009.

Results: Results from 270 NHs in 16 European countries are available. A median of 9.7% (11.7% mean) of all NH residents ($n = 26,063$) was diagnosed with an undefined wound. Among residents treated with ABs ($n = 1734$), 24% had a wound. However, when considering wound- and AB prevalence (dichotomized above and below the median) at institutional level there was no significant relation ($p = 0.19$). Of the AB using residents 2.5% was treated (i.e. prophylactic, empirical or documented) for a wound infection (WI). Among AB using residents with wounds 7% was treated for a WI. Remarkable is that 1.5% of the residents reported not to have wounds was treated for a WI. Of the AB treated NH residents that were admitted to a hospital within the last three months, 35% had a wound. Of those without hospital admission, 20% had a wound ($p < 0.0001$). *Escherichia coli* accounted for 47% of the microorganisms (MOs) detected among residents without a wound, in contrast to only 24% ($p = 0.0002$) for residents with a wound. Methicillin-resistant *Staphylococcus aureus* was found among 15% of residents with a wound, and 4% of those without ($p = 0.00015$).

Residents with a wound received more often empirical (55%) or documented AB treatments (22%) than residents without a wound (49%, $p = 0.045$ and 15%, $p = 0.0012$, respectively). Prophylactic ABs were used less for patients with (22%) than for those without wounds (36%, $p < 0.0001$). Residents with in contrast to those without wounds received more often combinations of penicillins and β-lactamase inhibitors (J01CR, 21% vs. 13%, respectively, $p < 0.0001$) and cephalosporins (J01D, 13% vs. 7%, $p = 0.012$). On the contrary, residents with wounds received other antimicrobials (J01X) significantly less (18% vs.31%, $p < 0.0001$). Finally, residents with compared to those without wounds received less often oral ABs (83% vs. 93%, respectively, $p < 0.0001$) but significantly more often parenteral ABs (15% vs. 7%, $p < 0.0001$).

Conclusion: The presence of wounds does not seem to significantly influence AB prevalence. However, there is a relation between the presence of a wound and recent hospital admission, registered MOs, AB indication, type of AB and route of administration.

O473 Antibacterials for systemic use in Belgian hospitals

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Objectives: The aim of the present study is to explore differences in antimicrobial consumption in Belgian hospitals including differences between ward types. This report was restricted to antibacterials for systemic use (WHO-ATC classification J01C).
Methods: Belgian hospitals were invited to report their antimicrobial use to the federal Scientific Institute of Public Health (IPH), which was responsible for the data collection, conversion into defined daily doses (DDD), analysis and feed back. The data collected were split up for non-pediatric and pediatric wards. There is also an optional reporting of intensive care wards and hematology-oncology wards.
Results: The overall results are shown in Table 1. The optional reporting was highly variable over different unit types (range of unit types:8–44). The median antibacterial use per bed-day was more than twice as high in ICU compared to NPD (1150 vs. 456 DDD/1000 bed-days for 2006 and 1209 vs. 536 DDD/1000 bed-days for 2007). The median antibacterial use in HAO (787 DDD/1000 bed-days in 2006, 943 DDD/1000 bed-days in 2007) was situated in between the use on NPD and HAO. For 2007, a big increase in variation in ICU was observed.
Conclusion: Overall incidence of antimicrobial use slightly increased during the observation period, possibly biased by the different number of participating hospitals in the second year of observation. The methodology allows a close trend follow up, with the possibility to compare the consumption between different unit types over time. From 2008 onwards, participation is obliged for the majority of Belgian hospitals.

Table 1. Hospital use expressed as DDD/1000 bed-days (subgroup J01)

		2006 (n = 28)		2007 (n = 55)	
Class	ATC	Mean	Range	Mean	Range
Beta-lactam antibacterials, penicillins	J01C	234	168–375	253	100–380
Other beta-lactam antibacterials	J01D	109	39–175	114	17–244
Quinolone antibacterials	J01M	65	8–102	67	7–115
Other antibacterials	J01X	35	12–61	37	10–81
Macrolides, lincosamides and streptogramins	J01F	23	8–38	25	7–64
Aminoglycoside antibacterials	J01G	14	1–38	14	1–55
Sulfonamides and trimethoprim	J01E	8	1–22	9	1–29
Tetracyclines	J01A	3	0–13	3	0–44
Amphenicols	J01B	0	0–1	0	0–3
Total		492	344–694	524	242–792

O474 Increased antibiotic use in Swedish intensive care units, 1999–2008

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The ICU-Strama programme was developed ten years ago and used for regular audit of antibiotic use, antibiotic resistance and infection control procedures in Swedish ICUs. It is a joint project between the ICU-Strama and the Swedish Intensive Care Registry. A central component has been a web-based application which includes a system for automatic feed-back. The purpose of this report is to increase awareness of the usefulness of the programme and provide a ten year trend analysis of antibiotic consumption.
Material and Methods: The data on antibiotic consumption based on the anatomical therapeutic chemical (ATC) classification system were collected from 64 Swedish ICUs and entered into the database using the web application. Antibiotic consumption was expressed as defined daily doses (DDD) per 1,000 occupied bed day (DDD1000). We used the annually updated DDD calculated by the WHO Collaborating Centre for Drug Statistics Methodology as the average maintenance dose per day in adults for the main indication of the drug(http://www.whocc.no/atcddd). Data were analysed using the non-parametric test for trend across ordered groups and Spearman's rank correlation using STATA/SE 9.2(StataCorp LP, College Station, TX, USA) and SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). Statistical significance was assumed if $P < 0.05$.
Results: The median antibiotic consumption increased from 1,256 DDD1000 1999 to 1,434 DDD1000 2008 ($p < 0.001$). Antibiotic consumption varied widely between different units during 2008, ranging between 696 and 2,722 DDD1000 with a median of 1,434 DDD1000. Trend analyses of usage of different classes of antibiotics were performed and showed increased carbapenem, triazole and piperacillin–tazobactam consumption ($p < 0.001$). There was no significant change in consumption of cephalosporins for the ten year period but a trend towards decreased consumption the last two years. No significant correlation between antibiotic consumption and standardised mortality rate was shown.
Conclusion: The high antibiotic consumption concurs with figures from European and US ICUs in general, but like a few ICUs in our programme, relatively low antibiotic consumption has been reported from Switzerland. The lower antibiotic consumption suggests that it is possible to reduce antibiotic consumption in the critically ill, but it has to be accompanied with a quality control system to make sure that it does not compromise patient outcomes.

O475 Developing a database to facilitate the assessment of antimicrobial consumption in an acute tertiary hospital

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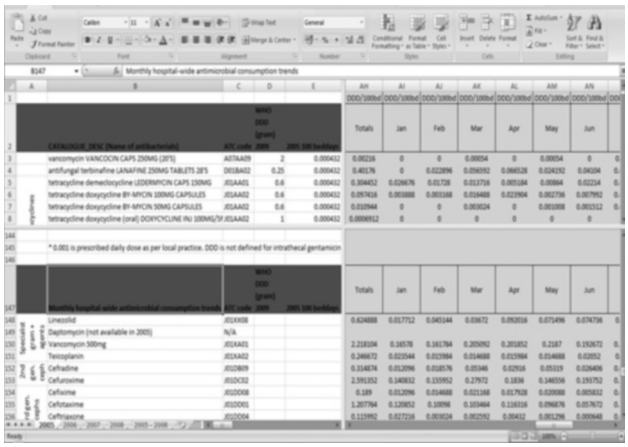
Background: Monitoring antimicrobial consumption trends is part of an effective systematic surveillance programme to prevent and control healthcare-associated infection and antimicrobial resistance. Expressing antimicrobial consumption data in the World Health Organization-recommended measurement unit – Defined Daily Dose (DDD) per 100 bed-days, is important for benchmarking purposes. This is also a part-fulfilment of the national guidelines of antimicrobial stewardship for hospitals in Ireland.
Objectives: To achieve this aim, the following have been carried out: (1) a national survey on the opinions of pharmacists on data collecting for antimicrobial consumption in hospitals, (2) development of an antimicrobial consumption database (2005–2008), expressed in DDD/100 bed-days, for hospital-wide and individual group of speciality wards/units, (3) evaluation of the antimicrobial consumption trends obtained from the database.
Methods: The original data presented in 'unit dose' is obtained from the Hospital's information database called the Diver Solution™, which can interface with Microsoft® Excel 2007. The aggregate in-hospital consumption data that includes both issues from and returns to pharmacy from 2005 to 2008 were collected. The ABC Calc. (version 3.1, 2006) was adapted in an Excel worksheet to create the antimicrobial consumption database, converting consumption data from 'unit-dose' to DDD/100 bed-days.



Results: The survey found a variation in the method of data collecting for antimicrobial consumption among hospitals. The lack of an appropriate pharmacy information technology and inadequate education and training on antimicrobial consumption data collection and reporting are some

of the main issues of concern. Results from the evaluation of the consumption trends demonstrate the need to monitor hospital-wide and individual speciality wards/units separately. Limitations of the ATC/DDD system have been identified and taken into consideration when interpreting the results.
Conclusion: Variation exists in the methods of collecting and reporting for antimicrobial consumption. The study has shown that setting up of antimicrobial consumption databases expressed in DDD/100 bed-days can be done at a local level. An antimicrobial consumption database should be available in all hospitals to facilitate the close monitoring of antimicrobial consumption, frequent feedback reporting to the prescribers and also to supplement the surveillance of microbial resistance.

O476 Antibiotic prescribing in outpatients: hospital and seasonal variations in Ujjain, Madhya Pradesh, India

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Objective: To explore seasonal patterns of antibiotic prescribing for selected infectious disease complaints for children up-to 5 years and adults (>18 years) reporting to out patient clinics of two hospitals in Ujjain.
Methods: This was cross sectional study; during 15 months period from 15th November 2007 to 15th February 2009. It covered 4 seasons, 2 winters, one summer and one rainy season. First consultations of all patients for suspected infectious aetiology at outpatients of two hospitals (one for profit and other academic) were included. A diagnosis prescribing form was filled by the treating consultant for each patient irrespective of whether an antibiotic was prescribed or not. An average of 70% of available consultants participated. Each prescribed antibiotic was coded according to the WHO Collaborating Centre for Drug Statistics Methodology, ATC classification index with DDDs 2009. All DDDs were calculated/1000 patients/diagnosis (DDD/TPD). Stata 10.0 was used for appropriate statistical tests.

Table. Number of patients per diagnosis, prescription rate with commonest prescribed antibiotic groups with Defined Daily Doses/ 1000 patients/diagnosis (DDD/TPD) per group

Diagnosis	Number of patients	Overall prescription rate %	Name and DDDs/TPD of commonest antibiotic group/antibiotic
URTI†	728	38.6	Quinolones 435.8 Levofloxacin 264
Ear discharge	105	96.2	Co-amoxiclav 152.7
Pneumonia	422	89.4	Quinolones 1889 Levofloxacin 1597.8
Vaginal discharge	1361	72.2	Metronidazole 11632.5 Doxycycline 9183 Quinolones 434.2
UTI‡	647	96.1	Quinolones 3647.2 Metronidazole 2893
Diarrhoea	525	53	Imidazole group 713 Co-trimoxazole 587.6 DUs/TPD
Dysentery	69	97.1	Quinolones 2118.6 Imidazole group 863
SSTI*	575	92.4	Co-trimoxazole 4032.5 DUs/TPD Quinolones 1873.4

†URTI: Upper respiratory tract infection; ‡UTI: Urinary tract infection; *SSTI: Skin and soft tissue infections.

Results: Out of a total of 5,733 patients antibiotics were prescribed in 3,732 (66.3%). These prescriptions contained 1–3 antibiotics, with a mean of 1.28 antibiotics per prescription. Indications were respiratory tract infections (32.2%), vaginal discharge (26.3%), urinary tract infections (16.3%), skin and soft tissue infections (15%), diarrhoea (9.2%) and prophylaxis (1%). Quinolones were the commonest group prescribed. Antibiotic prescribing was 37% less in academic hospital ($P < 0.001$). Prescribing peaked in rainy season with 70% of patients prescribed antibiotics. The independent predictors of antibiotic prescribing were seasons (2nd winter > 1st winter), facility (for profit > academic), age groups (adults > children), education level (illiterate > more educated), productive cough at presentation, ear discharge, pneumonia and dysentery. The number of patients per diagnosis, prescription rate with commonest prescribed antibiotic group with DDDs/ TPD per group is shown in table.
Conclusions: Statistically significant association between antibiotic prescribing and seasons, for profit hospital, age groups, education level, symptom of productive cough at presentation, ear discharge, pneumonia and dysentery was found. High use of quinolones is a cause of concern.

O477 Systemic antifungal therapy in European hospitals. Data from the ESAC point prevalence surveys 2008 and 2009

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Objectives: To determine the variability of antifungal treatment in European hospitals and to identify targets for quality improvement in antifungal prescribing.
Methods: The European Surveillance of Antimicrobial Consumption (ESAC) Point Prevalence Survey (ESAC-PPS) was carried out during a maximum of two weeks in 50 European hospitals in 2008 and in 134 hospitals in 2009. A web-based application was developed for online data entry by the hospitals. Antimycotic prescriptions were recorded using the WHO ATC classification including 'antimycotics for systemic use' (J02) and terbinafine (D01BA02). Demographic data on treated patients, indications, and diagnoses were collected.
Results: From a total of over 85,000 admitted patients, 25,201 (29%) received antimicrobials. Patients receiving antifungals amounted to 1,309 (3.8% of all antimicrobials) receiving a total of 3,125 therapies (mean 2.4, range 1–7). Dual therapy was used in 487 (37%) patients, and triple therapy in 24% of patients. The most commonly prescribed antifungal was fluconazole, accounting for 60% of all antifungal therapy followed by caspofungin (10%). The most frequently used antifungal-antibacterial combinations included fluconazole plus either a quinolone or a β-lactam, mainly for medical prophylaxis. The proportion of parenteral use within the total antimycotic prescriptions was 47%. The oral route accounted for 60% of fluconazole prescriptions. In 38% of cases the site of infection was undefined whilst the most common sites were respiratory (20%), gastro-intestinal (16%) and ENT (4%). The medical specialty accounted for the majority (69%) of antifungal use. Hospital acquired infections represented 46% of all the indications followed by medical prophylaxis at 30%.
Conclusion: These ESAC-PPS results showed minimal variation in treatment for fungal infections. This was mainly observed in the predominance of fluconazole. However, high use of fluconazole can increase the prevalence of other fungi, e.g., *Candida glabrata*, and therefore increase the need for newer antifungals which are active against inherently resistant pathogens. Ongoing surveillance will enhance efforts to limit the extent of antifungal use and resistance. Antifungal prophylaxis in the immunocompromised host needs further exploration. ESAC-PPS methodology is the right tool for such analysis.

O478 European Surveillance of Antimicrobial Consumption: outpatient systemic antiviral use in Europe

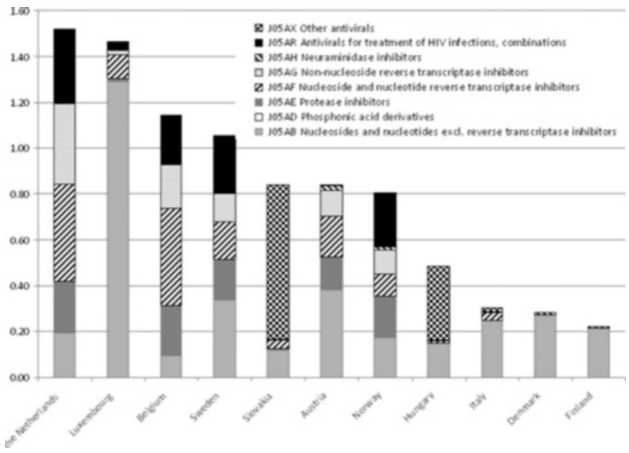
N. Adriaenssens, S. Coenen, A. Muller, V. Vankerckhoven, H. Goossens on behalf of the ESAC Study Group*

Objectives: To assess the total outpatient systemic antiviral use in Europe and to identify the antiviral substances most commonly used before the outbreak of the A/H1N1 pandemic as a historical reference.
Methods: The European Surveillance of Antimicrobial Consumption (ESAC; www.esac.ua.ac.be) project, now funded by the European Centre for Disease Prevention and Control (ECDC; agreement number 2007/001), continues to collect data on antimicrobial consumption for all Member States, candidate countries and European Free Trade Association-European Economic Area countries using the anatomical

therapeutic chemical (ATC) classification and the defined daily dose (DDD) measurement unit. For 2007, data on outpatient use of all antivirals for systemic use (ATC J05), aggregated at the level of the active substance, was collected and use was expressed in DDD (WHO ATC/DDD, version 2008) per 1000 inhabitants per day (DID).

Results: Total outpatient systemic antiviral use in 2007 in 11 European countries varied by a factor of 6.9 between the country with the highest (1.5 DID in the Netherlands) and the country with the lowest (0.2 DID in Finland) use. In most countries substances to treat HIV infection (ATC J05AE, J05AF01–07, J05AF09, J05AG, J05AR, J05AX02, J05AX05 and J05AX07–09) represented more than 50% of the total outpatient systemic antiviral use. In Finland, Denmark, Italy and Luxembourg nucleosides and nucleotides excluding reverse transcriptase inhibitors (ATC J05AB) represented more than 80% of the total outpatient antiviral use. The use of neuraminidase inhibitors (ATC J05AH) was the highest in Austria (0.02 DID) and varied from 3.42% in Denmark to no use reported in Belgium.

Conclusion: Our study demonstrates a variation of outpatient systemic antiviral use in Europe as striking as that of outpatient systemic antibiotic, antimycotic and antifungal use. More in-depth data on outpatient systemic viral use from more countries are needed to explain this variation. The ESAC data facilitate auditing of antiviral prescribing and evaluation of the implementation of guidelines and public health policies e.g. those related to A/H1N1.



Emergence and spread of resistance

O479 Emergence and spread of GES-type-expressing *Acinetobacter baumannii* in Belgian hospitals

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Objectives: Worldwide emergence and spread of multidrug resistant *A. baumannii* (AB) is a matter of concern. We studied the microbiologic, epidemiological and molecular characteristics of GES-like producing AB isolates (GPAB) recovered from 5 different Belgian hospitals.

Methods: Antibiotic susceptibilities were determined by agar disk diffusion, VITEK2, and Etest MIC determination. ESBL, carbapenemase coding genes and their genetic environment were analyzed by PCR-sequencing. OXA-51 and ADC-like coding genes were also characterized and the isolates were typed by PFGE. β-lactamase activities were analyzed by IEF.

Results: Between april 2009 and September 2009, 9 patients with GPAB isolates were identified in 5 Belgian hospitals. Isolates were recovered upon admission from wound infection, and one empyema. Index patients (pts) were transferred from Turkey (n = 2), Egypt (n = 2) and Gaza Strip (n = 1). One pt was at the origin of a nosocomial outbreak (n = 4 pts) in a burn unit requiring the closure of the unit for outbreak control. Overall 3 pts dead but no attributable mortality to GPAB was observed. Not common origin could be established between the index pts of the 5 hospitals neither by epidemiological data nor PFGE analysis. Phenotypic resistance profile was difficult to distinguish from OXA-carbapenemase producing-AB. All isolates showed multiresistance including carbapenem (MICs to imipenem and meropenem ranging from 12 to >32 mg/L). Doripenem showed better activity with MICs from 3 to 32 mg/L. Only colistin remained susceptible in all isolates. Three different alleles (blaGES-11 from Turkey, Gaza and Egypt; blaGES-12 from Egypt and a new blaGES-14 from Turkey) were detected as part of an identical Class I integron including also aac6′Ib and dfrA7. No other ESBL or carbapenemase genes were detected except OXA-51-like which was present in each isolate but not downstream to ISAba1. By IEF, a band inhibited by clavulanic acid and corresponding to GES was visualized at pI of about 6.

Conclusion: AB harboring blaGES-11 and blaGES-12 alleles were detected recently in France and in UK but were not yet reported elsewhere. The blaGES-14 variant is reported here for the first time in a pan-resistant AB isolate. This event further highlights the potential of acquisition of resistance genes by AB and the risk of travel import of multi-resistant bacteria with the possible subsequent inter/intra-hospital spread in acute care hospitals.

O480 Resistance in agents of hospital-acquired respiratory infection in the United Kingdom and Ireland, 2008–2009

R. Reynolds, I. Morrissey, R. Hope on behalf of BSAC Working Party on Resistance Surveillance*

Objective: Hospital-acquired respiratory infections are an important source of morbidity, are associated with substantial mortality, and are caused by a wide range of pathogens potentially harbouring diverse antimicrobial resistance mechanisms. The BSAC Respiratory Resistance Surveillance Programme now monitors these infections.

Methods: Isolates obtained more than 48 hours after hospital admission (H48) were considered to be hospital-acquired. In 2008/09, 22 laboratories in the UK and Ireland sent up to 13 H48 lower respiratory isolates each of *S. aureus* and *Pseudomonas* and 50 Enterobacteriaceae for central MIC testing by BSAC methods. Preliminary results for isolates tested up to October 2009 were compared with those for H48 blood isolates from the similar 2008 BSAC Bacteraemia Resistance Surveillance Programme.

Organism (>48 hours after hospitalization)	Source	N	% non-susceptible				
			CIP	CTX	ERY	GEN	TET
MRSA	LRTI	77	96	–	82	4	9
	blood	72	92	–	75	6	7
MSSA	LRTI	94	20	–	22	1	1
	blood	159	18	–	18	3	4
E. coli	LRTI	285	26	15	–	12	–
	blood	217	22	12	–	11	–
Klebsiella	LRTI	209	14	15	–	9	–
	blood	117	19	18	–	10	–
Enterobacter	LRTI	156	15	41	–	7	–
	blood	107	16	35	–	9	–

Results: MRSA were more prevalent in H48 respiratory *S. aureus* (77/171, 45%) than in H48 blood *S. aureus* (72/231, 31%), but had a similar non-susceptibility profile. Of 796 H48 respiratory Enterobacteriaceae, 37% were *E. coli*, 28% *Klebsiella*, 20% *Enterobacter* and 14% other genera, compared with estimates of 59%, 18%, 9% and 14% respectively among H48 blood isolates (combined data, BSAC and HPA voluntary surveillance system 2008). Thus, *Klebsiella* and especially *Enterobacter* were more common, and *E. coli* less common, in respiratory compared with blood infections. Non-susceptibility profiles were similar for respiratory and blood isolates. Non-susceptibility rates among 169 H48 respiratory *P. aeruginosa* were modest and comparable

with those of 125 H48 blood isolates for ceftazidime (3 vs. 4%), gentamicin (5 vs. 6%) and piperacillin–tazobactam (8 vs. 10%), but clearly higher for imipenem (21 vs. 5%) and perhaps also ciprofloxacin (24 vs. 14%, but with many MICs close to the breakpoint).
Conclusion: Resistance rates in hospital-acquired lower respiratory isolates are broadly similar to those in bacteraemia, but may differ substantially in some cases. More data and analysis are needed to clarify the influence of factors such as patient age and treating speciality on these apparent differences in resistance between respiratory and blood isolates. Direct surveillance of resistance in specific infections is preferable to extrapolation from data obtained in bacteraemia, when possible.

O481 Antimicrobial resistance among invasive *Streptococcus pyogenes* isolates in Portugal

A. Friaes, M. Ramirez, J. Melo-Cristino (Lisbon, PT)*

Objectives: Although *S. pyogenes* is universally susceptible to penicillin, in the treatment of complicated Group A Streptococci (GAS) infections such as necrotizing fasciitis or STSS, the association of penicillin with clindamycin has been advocated. Resistance to the latter has been described in GAS, associated with resistance to macrolides. The aim of this work was to evaluate the resistance of GAS invasive isolates to several antimicrobial agents and to further characterize the macrolide resistance isolates.
Methods: A total of 306 invasive GAS isolates recovered in Portugal during 2000–2008 were tested for susceptibility to penicillin, vancomycin, erythromycin, tetracycline, levofloxacin, chloramphenicol, clindamycin, quinupristin-dalfopristin, and linezolid by disk diffusion. Intermediate susceptibilities were confirmed by MIC determination using E-test strips. Resistance genotypes were determined by PCR.
Results: All the 306 GAS isolates were susceptible to penicillin, vancomycin, chloramphenicol, quinupristin-dalfopristin, and linezolid, and only two (0.7%) presented reduced susceptibility to levofloxacin (MIC = 3 and 4 ug/ml). A total of 32 isolates (10.5%) were resistant to erythromycin. Of these, 20 were also constitutively resistant to clindamycin (cMLSB phenotype, 62.5%), whereas 12 presented the M phenotype (37.5%). The cMLSB phenotype was associated with the presence of the erm(B) gene, whereas the M phenotype was associated with mef(A). Non-susceptibility to tetracycline was found in 42 isolates (13.7%), of which 8 also expressed the cMLSB phenotype and 1 the M phenotype.
Conclusion: Erythromycin resistance among invasive GAS isolates in Portugal did not vary significantly during 2000–2008, contrarily to what has been reported for isolates causing pharyngitis, whose macrolide resistance decreased from 1999 to 2006. The macrolide resistance observed in this study for invasive GAS isolates (10.5%) is in line with reports from other countries, as well as with the overall rate reported for pharyngitis isolates in Portugal during 2004–2006 (13.2%).

O482 Effect of ertapenem on susceptibility of imipenem to *Pseudomonas aeruginosa* six years later

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Objectives: There is continued concern that ertapenem (E) will negatively effect imipenem (I) susceptibility to *Pseudomonas aeruginosa* (PA). Our purpose is to monitor E effect on (I) susceptibility to PA from 2003–2008. Other antipseudomonals on the formulary, piperacillin/tazobactam (P/T), cefepime (C), and tobramycin (T), were also examined.
Methods: Antibiotic susceptibilities to I, P/T, C, and T were determined by microdilution MIC using Microscan panels. E was not on Microscan panels until 2007, so Etest was used prior to 2007. Change in I susceptibility was analyzed using Mantel-Haenszel χ^2 test for linear trend. A Poisson model was used to estimate change in carbapenem defined daily doses/1000 patient days (DDD) over time. A rate ratio (RR) was calculated to compare carbapenem usage between 2003 and 2008 with a 95% confidence interval.
Results: *P. aeruginosa* susceptibility to I, PT, C, and T remained the same over 5 years and improved in year 6. An overall χ^2 test ($p = 0.010$) indicated a difference in percentages over time. The Mantel-Haenszel test for linear trend ($p = 0.004$) indicated an increasing trend in percentages over time. 91% ESBL *K. pneumoniae* and 100% ESBL *E. coli* and *K. oxytoca* isolates tested to E were susceptible MIC < 2. Total carbapenem use had significantly increased from 2002 (28DDD/1000PD) to 2008 (42.4DDD/1000PD) RR = 1.08 (95% CI, 1.01–1.15, $p = 0.017$). E was 13–44% of all carbapenem use; the increase was significant RR=1.24 (95% CI, 1.06–1.47, $p = 0.006$).
Conclusion: The use of ertapenem did not negatively effect the susceptibilities of imipenem, P/T, C, or T to PA. *P. aeruginosa* susceptibility to imipenem, PT, C and T remained the same or improved over these 6 years.

Organism	Susceptibility (No. of organisms tested)					
	2003	2004	2005	2006	2007	2008
K. pneumoniae	99% (537)	99% (542)	99% (703)	99% (718)	99% (699)	100% (694)
Kp. ESBL	95% (40)	100% (29)	99% (135)	100% (132)	100% (150)	99% (85)
E. coli	100% (1026)	100% (1047)	100% (1451)	100% (1833)	100% (1801)	100% (1653)
Ec. ESBL	100% (11)	100% (21)	100% (19)	100% (21)	100% (36)	100% (62)
E. cloacae	98% (211)	98% (187)	99% (260)	97% (321)	100% (339)	100% (296)
S. marcescens	98% (115)	98% (138)	99% (191)	100% (197)	100% (181)	98% (206)
P. aeruginosa	71% (741)	71% (819)	70% (923)	72% (1174)	72% (1229)	77% (1020)

O483 Growing role of community-acquired MRSA infections in the United States: a 10-year trend of replacement and expansion

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Objectives: To describe the role of Community-Acquired (CA) MRSA in inpatient and outpatient staphylococcus aureus infections over a 10 year period in the US.
Methods: The study used the TSN Network® surveillance database (Eurofins Medinet) for the period 1998 to 2007. The database contains information on isolate source, setting (ambulatory or hospital), geographic region, and demographic characteristics such as gender and age. Identical CLSI breakpoints were used for all the time points. CA-MRSA phenotype was defined by a resistance profile that includes the absence of gentamicin/cotrimoxazole resistance, and the absence of ciprofloxacin/ clindamycin/ erythromycin co-resistance. Using multivariate logistic regression, we computed adjusted phenotype prevalences and odds ratios (OR) with 95% confidence intervals (CI).
Results: The study consisted of 824,307 *S. aureus* isolates. MRSA prevalence continuously increased over the 10 year period from 32.7% in 1998 to 53.8% in 2007 (OR 2.4, 95% CI 2.3–2.5). CA-MRSA represented an increasing proportion of MRSA from 22.3% in 1998 to 66.1% in 2007 (OR 6.7, 95% CI 6.5–6.9). Changes in CA-MRSA prevalence were observed for all age-groups, isolate sources, inpatient or outpatient settings, and across all geographic regions of the US. However, a comparatively larger statistically significant temporal increase of CA-MRSA occurred in children and young adults as compared to the elderly, and in abscess and wound isolates when compared to blood and sputum. By 2007, 81.5% of all MRSA isolates were categorized as CA-MRSA among children, while CA-MRSA represented 48.9% of MRSA isolates from the elderly. In 2007, out of all MRSA, CA-MRSA represented 85.7% of all abscess, 75.4% of wound, 43.6% of blood and 30.4% of

sputum isolates, as well as 84.5% of outpatient and 40.8% of inpatient isolates.

Conclusion: In the study period, MRSA isolates have become a growing proportion of all *S. aureus* isolates. The majority of the growth of MRSA share of *S. aureus* infections is explained by CA-MRSA, which has not only proportionally expanded but also replaced HA-MRSA. These changes occurred principally among abscess and wound isolates, children and young adults, but with a lesser degree also among other isolate sources and age groups. Four out of five isolates in the outpatient setting now possess the CA-MRSA phenotype. These findings may have preventive and therapeutic implications in the management of *S. aureus* infections.

O484 Resistance of staphylococci isolated from infected hip arthroplasties in Norway

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Objectives: Antibiotic prophylaxis is commonly used during prosthesis surgery both systemically and locally in cement. We have examined bacterial findings from specimens taken during revision due to deep infection reported to the Norwegian Arthroplasty Register (NAR). Susceptibility to aminoglycosides (gentamicin, tobramycin and netilmicin) and βlactamase-stable penicillins (meticillin, oxacillin and cloxacillin (the methicillin-group) is presented. These antibiotics are the most commonly used in prophylaxis and in treatment of prosthetic joint infections (PJI).

Methods: We collected bacterial findings from operations reported as revisions for infection to NAR. The information was collected from notes from the ten hospitals in Norway that reported most revisions for infection from 1987 to 2007. In this period 730 revisions were reported to NAR from these ten hospitals. The total number of reported revisions was 1443 from all Norwegian hospitals. In this study we included operations with one or more positive sample. The bacteria were tested against different antibiotics at different hospitals and in different time periods. We used the χ^2 test for linear trend to evaluate changes in distribution of sensitive, intermediate and resistant (S, I, R) bacteria over time. We excluded the first five-year period due to low number of cases compared to the latter three five-year periods (5 vs. 31, 38 and 70, respectively).

Results: The most frequent bacteria isolated were coagulase-negative staphylococci (CoNS) (37%), and *S. aureus* (SA) (17%). Overall 153 operations with CoNS and 74 with SA were found.

Among CoNS 53% of the bacteria were resistant to aminoglycosides. The proportion of resistance increased from 35% in 1993–1997 to 43% in 1998–2002, and to 55% in 2003–2007 ($p = 0.1$). The overall resistance of CoNS to the meticillin-group (MRSE) was 62%. We found that 45% were MRSE in 1993–1997. The proportion increased to 50% in 1998–2002 and to 76% in 2003–2007($p = 0.002$).

All *S. aureus* were sensitive both to aminoglycosides and to the methicillin-group.

Conclusion: The proportion of MRSE was increasing with time in the present study. There was also a tendency to increased resistance to aminoglycosides among CoNS. No MRSA was found.

This study shows that the development of antibiotic resistance is an increasingly important challenge in the management of PJI.

O485 Increasing *in vitro* antibiotic resistance against *Pseudomonas aeruginosa* isolates from patients in the intensive care unit over the last two years

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Objectives: *Pseudomonas aeruginosa* is considered a "high-risk" pathogen responsible for severe intensive care unit (ICU) – acquired infections. In an era of increasing resistance, nosocomial infections caused by *P. aeruginosa* are very common despite the application of preventive measures in ICU leading to prolonged hospital stay, increased morbidity, mortality and treatment cost. Very often, in the last years, clinicians are left with very few antimicrobials as therapeutic options. Therefore, we aimed to study the susceptibility profile of *P. aeruginosa* clinical isolates against an extended panel of antibiotics in the last 2 years.

Methods: Beginning January 2008 to October 2009 all strains of *P. aeruginosa* isolated from surgical patients in the ICU were studied. In order to identify aerobic microorganisms we inoculated the specimens on blood agar, MacConkey, Mannitol Salt and Sabouraud Dextrose agar, and then incubated the plates at 37º C for 24 hours, whereas anaerobic cultures were carried out on Wilkins-Chalgren agar at 37º C for 48 hours. The identification of isolated strains and their susceptibility test to antibiotics using an extended panel of antibiotics were carried out with the automated system VITEK 2 (bioMérieux, Marcy l'Etoile, France).

Results: A total of 52 isolates from 21 patients in 2008 and 49 isolates from 19 patients in 2009 were studied. An increased resistance in all antimicrobials studied (except for colistin), was observed. The most active antimicrobials against *P. aeruginosa* isolates during 2008 and 2009 were piperacillin with 15% and 32% resistance, respectively, piperacillin/tazobactam 8% and 17%, gentamicin 21% and 50%, amikacin 18% and 43%, tobramycin 18% and 47%, ceftazidime 29% and 46%, and ciprofloxacin 26% and 49%, respectively. Thirty-six percent of the isolates were resistant to imipenem in 2008 and 37% in 2009 while for meropenem the resistance was 31% and 40%, respectively, and for tigecycline 36% and 58%, respectively. Finally, colistin was the least resistant among all antimicrobials tested (9% in 2008 and 2% in 2009).

Conclusion: The development of resistance must be carefully monitored in hospitals. Due to an increased use of tigecycline in our ICU, an emerging resistance to this new and promising antimicrobial was observed. Colistin maintained significant *in vitro* activity against *P. aeruginosa* isolates and should be considered in critically ill patients with difficult-to-treat infections.

O486 Surveillance of drug resistance of *Mycobacterium tuberculosis* patterns in clinical sputum samples

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Introduction: Testing for first line anti tuberculosis drugs formed the back bone of the study based on routine drug susceptibility testing since these drugs are widely used in the world and tested for in rounds of proficiency testing among reference laboratories. Therefore the need to determine the susceptibility and resistance patterns of *Mycobacterium tuberculosis* in 641clinical samples for diagnosis and from retreatment samples in the period between 2000 and 2009.

Objective: To determine the drug resistance patterns for *Mycobacterium tuberculosis* in clinical samples.

Methodology: This was a retrospective descriptive study conducted at chest diseases laboratory. 641 Tuberculosis positive cultures by proportion methods. Data of the drug resistance survey conducted during this period was excluded.

Results: The total number of strains examined was 641, showing drug resistance to one, more drugs in *Mycobacterium tuberculosis* strains.

Total resistant strains 228 (31.7%), Mono resistance to Streptomycin 54 (8.4%), Isoniazid 23 (3.5%), Rifampicin 7 (1.09%), Ethambutol 4 (0.04%). Resistance to two drugs Rifampicin and Isoniazid 41 (6.3%), Streptomycin and Isoniazid 11 (1.7%), Streptomycin and Rifampin 12 (1.8%), Isoniazid and Ethambutol 3 (0.46%). Resistance to three drugs Streptomycin + Isoniazid + Rifampicin 11 (1.7%), Isoniazid +Rifampicin +Ethambutol 3 (0.46%), Streptomycin + Isoniazid + Ethambutol 1 (0.15%) and resistance to all four drugs included 15 (2.3%).

Discussion: The trend seen in the study period shows that proportions of resistance to Rifampcin and Isoniazid was increasing suggestive of secondary drug resistance.

The resistance to streptomycin was high at 8.4%. Isoniazid resistance trend was at 3.5%. Ethambutol 0.04%.

Conclusion: out of 641 positive cultures tested for drug susceptibility, 228 were resistant to one, two, three and four drugs. Multi drug

resistance (6.3%) showed a rising trend and threatening to be a problem. Streptomycin resistance was highest (8.4%) may be because it is used for treatment of other infections. Mono resistance to Isoniazid (5.1%) was in rising proportions due to its use in preventive administration. Resistance to Rifampicin (1.09%), Ethambutol at 0.47% respectively.

O487 Four major drug-resistant mycobacterial strains isolated between 2001–2008 in Konya, Turkey

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Objectives: In our study, we aimed to detect the presence of *M. tuberculosis* with the BACTEC 460TB system (2001–2006) and the BACTEC MGIT 960 (Mycobacteria Growth Indicatör Tubes, Becton-Dickenson) system (2007–2008) in clinical samples containing suspected pulmonary and extrapulmonary tuberculosis and also to determine the resistance of the isolated *M. tuberculosis* strains against four major antimicrobial drugs. The study period was between 2001 and 2008.
Methods: From 2001 to 2006, antimicrobial susceptibility tests to isoniazid (INH), streptomycin (SM), ethambutol (EMB) and rifampin (RIF) on 635 Mycobacterial strains were performed using the radiometric BACTEC 460TB system. From 2007 to 2008, antimicrobial susceptibility tests on 270 Mycobacterial strains were performed with the BACTEC MGIT 960 SIRE kit.
Results: Of the 1039 M.tbc spp., antibiotic susceptibility tests were performed on 905. We observed that 84.8% (767/905) were susceptible to all 4 antibiotics, 15.2% (138/767) were resistant to at least one of the 4 antibiotics. Mono resistance rates to INH, SM, RIF, and EMB were 4.4, 1.3, 0.6 and 0.4, respectively. Total monodrug resistance was observed in 6.7% of 905 the Mycobacterial isolates. Resistance to two drugs was observed in 48 (5.3%) isolates, to three drugs in 24 (2.7%) isolates and to four drugs in 5 (0.5%) isolates. The prevalence of MDR (Multidrug Resistance) in 905 Mycobacterial isolates was 4% (36/905).
Conclusions: According to these results it could be concluded that, drug resistance which requires immediate solution, continues to be a major problem in our region.

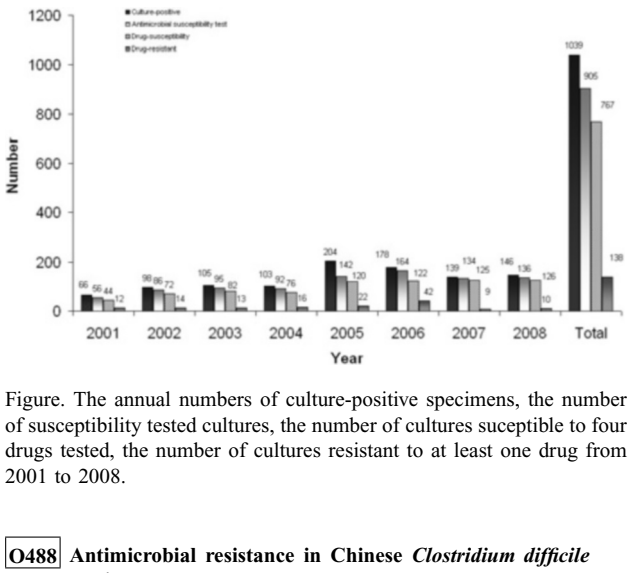

Figure. The annual numbers of culture-positive specimens, the number of susceptibility tested cultures, the number of cultures suceptible to four drugs tested, the number of cultures resistant to at least one drug from 2001 to 2008.

O488 Antimicrobial resistance in Chinese *Clostridium difficile* strains

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Objectives: *Clostridium difficile* infection is the leading cause of nosocomial diarrhea. The emergence and spread of resistance in *C. difficile* are complicating the treatment and prevention. The purpose of the present study was to investigate the antimicrobial susceptibility patterns and resistance mechanisms of Chinese *C. difficile* strains.
Methods: 110 toxigenic *C. difficile* isolates collected between December 2008 and May 2009 at Fudan University Hospital Huashan were analyzed for their antibiotic susceptibility patterns using the agar dilution method. The heteroresistance to metronidazole in fresh isolates were detected by disc diffusion and Etest methods. Resistance molecular basis was investigated using polymerase chain reaction (PCR) and DNA sequencing.
Results: 16 different PCR ribotypes were identified with a dominant clone 017 accounting for 37.3% of the isolates, followed by 001 and H. Ribotype 027 was not found but one isolate belonged to ribotype 078. All the isolates were susceptible to vancomycin and piperacillin/tazobactam. However, 18 of 78 (23.1%) isolates were found to be transient heteroresistant to metronidazole. Resistance to moxifloxacin, ciprofloxacin, levofloxacin, erythromycin, clindamycin, tetracycline, rifampin, rifaximin and fusidic acid was found in 61.8%, 100%, 66.4%, 85.3%, 88.1%, 62.7%, 29.1%, 29.1% and 8.2% of the isolates, respectively. The isolates of common PCR ribotypes were more resistant than the uncommon ribotypes. The prevalence of resistance genes and mutations among the resistant isolates was as follows: ermB, 69.1%; tetM, 97.1%; gyrA mutation, 63.2%; gyrB mutation, 4.4%; gyrA and gyrB mutation, 32.4%, rpoB mutation, 100%, respectively. The fusA mutation was only found in one isolate with minimum inhibitory concentration (MIC) of 4 mg/L.
Conclusions: Many *C. difficile* isolates now show an alarming pattern of resistance to the antimicrobial agents used in China. Isolates of common PCR ribotypes are more resistant than uncommon ribotypes, especially the dominant strain 017. Most of the resistance mechanisms which have been identified in *C. difficile* are similar to those in other Gram-positive bacteria.

Molecular architecture and antigenic structure of flaviviruses

K494 Molecular architecture and antigenic structure of flaviviruses

F. Heinz, K. Stiasny, H. Holzmann, S. Kiermayr (Vienna, AT)*

The genus Flavivirus in the family Flaviviridae comprises about 70 different virus species, many of which are arthropod-borne and transmitted to their vertebrate hosts by mosquitoes or ticks. Among those, the most important human pathogens are yellow fever virus, dengue viruses types 1 to 4, Japanese encephalitis virus, West Nile virus and tick-borne encephalitis virus. Depending on specific and different natural host systems, these viruses differ with respect to their areas of geographical distribution but – at least in some instances – have the potential to emerge as new pathogens in previously non-endemic regions. Flaviviruses are positive-stranded RNA viruses that have a lipid envelope and only three structural proteins, designated C (capsid), M (membrane) and E (envelope). Non-infectious, immature particles – containing a precursor of the M protein (prM) – are formed intracellularly and the proteolytic cleavage of prM by the cellular protease furin generates mature and infectious virions shortly before their release from infected cells.
With respect to their structure, flaviviruses are among the best-studied enveloped viruses. The atomic structures of E proteins of several flaviviruses have been determined using X-ray crystallography, and the architectures of both immature and mature virions are known from cryo-electron microscopic studies. Through the combination of structural and immunological investigations, we have gained a detailed understanding of the effects of antibody-binding to the virus and its different antigens. This includes the definition of virus neutralization and antibody-mediated enhancement (ADE) of infectivity at a molecular level. The latter phenomenon has been proposed to play an important role in the immunopathology of severe forms of dengue virus infections (hemorrhagic dengue fever and dengue shock syndrome) in the course of sequential infections with different dengue serotypes. All of these structural insights have also shed new light on the different degrees of cross-reactivity between flaviviruses, including the existence of cryptic epitopes that are recognized by broadly flavivirus cross-reactive

antibodies without leading to neutralization. The current picture of antibody interactions with flaviviruses will be presented in the context of their biological significance.

Treatment of invasive aspergillosis: pharmacokinetics vs. resistance

S496 Clinical impact of azole resistance

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The class of the azoles have become the most prominent class of compounds for the management of invasive aspergillosis. The clinically licensed triazoles with activity against *Aspergillus* include itraconazole, voriconazole and posaconazole. The azoles are also the only drugs that can be administered orally. The azoles interact with the biosynthesis of ergosterol, which is an important component of the fungal cell membrane. Intrinsic resistance to azoles has been documented for *A. calidoustus,* but the vast majority of species are susceptible *in vitro*. Recently, *Aspergillus* species with acquired resistance to azoles have been reported, especially in *A. fumigatus*. It appears that resistance may develop during azole therapy, especially in patients with chronic therapy including patients with chronic infection and aspergilloma. Another route of resistance development may be exposure of *Aspergillus* to azole fungicides that are used in our environment. Patients would then inhale azole-resistant conidia and develop azole-resistant aspergillosis. The consequence of this route of transmission is that azole-resistant disease may occur in patients without previous exposure to azole compounds. Azole resistance is commonly due to mutations in the Cyp51A-gene and is associated with different phenotypes. Isolates may be resistant to a single azole compound, but more commonly a cross-resistant phenotype is observed. Several cases of azole-resistant aspergillosis have been reported and commonly these patients failed to respond to azole therapy. Azole-resistant *A. fumigatus* isolates remain virulent and are capable of causing invasive disease in patients at risk. The efficacy of azole compounds against azole-resistant isolates, with different resistance mechanisms, has been investigated in experimental models of invasive aspergillosis. These indicate that the minimal inhibitory concentration has major impact on the efficacy of the azole. Elevated MICs are associated with loss of efficacy of the azole compounds *in vivo*. The use of azoles should be avoided in patients with azole-resistant aspergillosis, if possible. Alternative agents such as lipid-formulations of amphotericin B or caspofungin appear to retain their efficacy against azole-resistant isolates. A significant problem is the early diagnosis of azole-resistance as in the majority of cases an isolate is not obtained and, if an isolate is cultured, *in vitro* susceptibility testing causes delay in treatment with effective agents.

Living in the ideal CM/ID world

S497 Searching for an ideal clinical microbiologist – ID perspective

J. van der Meer (Nijmegen, NL)*

Infectious diseases in our time pose a tremendous challenge to the medical community. We are confronted with emerging infectious diseases, increasing antimicrobial resistance, stagnant development of new antibiotics, poor quality of antimicrobial prescribing, poor compliance with hospital hygiene, a revolution in microbiology, increasing numbers of immuno-compromised and frail patients, and upcoming immunotherapeutic possibilities. To cope with these problems in a successful way, synergy between clinical microbiologists and clinicians with infectious disease expertise is needed more than ever.
In my view, the profile of the ideal microbiologist is that of a well-trained physician who received a specialty training which encompasses laboratory skills (microbiology, molecular biology), practical training in clinical infectious diseases, epidemiology and public health, hygiene, research and communication.
In the ideal setting, there is close partnership with the ID physicians and there is complementarity, optimal collaboration, mutual recognition and trust. In daily practice, however, obstacles may be encountered, such as competence, mistrust, ego and other psychological factors, finances, time and organizational constraints. Such hurdles should be recognized and dealt with.

S498 Searching for an ideal infectious diseases specialist – CM perspective

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The nature of infectious diseases (ID) has changed significantly over the past decades. Many ID have successfully been eradicated through vaccination and the course of some infections has changed due to improved standard of life. On the other hand, changes in life style and longer life expectancy have promoted the emergence and spread of new ID. In spite of rigorous attempts to control hospital care associated infections these infections are becoming a growing problem in modern medicine. The appearance of multiply resistant organisms and the shortage of new antibiotics in the pipeline have placed further demands on developing expertise in the difficult field of antibiotic stewardship. Therefore, the profession of an ID doctor in the 21st century is a very complex one. He or she should have good skills and knowledge in recognizing traditional infectious diseases even those that are believed to be eradicated or not frequent in the area of practice. However, over the past decades infectious diseases with typical clinical presentation and a well defined pathogen have been to a significant extent replaced by infections whose symptoms overlap with the symptoms of the underlying diseases in the same patient or can not be easily differentiated from other causes of systemic inflammatory response syndrome. Therefore an ID doctor has to have good communication skills in dealing with other colleagues of different specialties and should be used to work in a team, appreciating different skills brought by different disciplines. From a clinical microbiologist point of view an ideal infectious disease specialist should recognize that the field of clinical microbiology (CM) has undergone the same transformation from mostly research and public health oriented microbiology towards microbiology at the patient bedside. In many patients only individual approach and team work will produce satisfactory diagnosis and treatment of infection. The fact that skills and competencies of modern ID and CM doctors overlap to a greater extent than with other specialties makes a solid ground for a close collaboration and partnership between these two professions. This is important in the field of clinical practice as well as in the field of research. There is a growing number of literature related to infectious diseases and anyone involved in the management of such patients should have a basic knowledge in research methodology and be competent in practicing evidence based medicine.

Nocardia infections

S500 Clinical infection and treatment

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Nocardiosis is an uncommon bacterial infection with a wide variety of clinical manifestations in immunocompetent and immunocompromised patients. The number of cases reported in the literature is increasing. This might be due to an absolute increase in the number of immunocompromised patients but also to improvement in laboratory techniques to detect nocardiosis. Host resistance to nocardial infection depends on neutrophils in early lesions and then the cell-mediated immune response. The most common predisposing factors to opportunistic nocardia infections are long-term steroid usage, chronic obstructive pulmonary disease, neoplastic disease, and human immunodeficiency virus infection. Clinical manifestations of nocardiosis range from cutaneous infections caused by traumatic inoculation in normal hosts to severe pulmonary and central nervous system diseases in immunocompromised hosts. The genus of *Nocardia* is rapidly expanding

and the species distribution varies with different geographical locations. Previous studies also emphasized the importance of determining the species of *Nocardia*, because different species and isolates vary in their antimicrobial susceptibility patterns. Sulphonamides have been used in the treatment of nocardiosis since the 1940s and are still the drugs of choice and the most common antimicrobials used to treat these infections. Sulphonamide monotherapy, however, was associated with mortality rates of almost 50% for patients with central venous system (CNS) nocardial infections. In addition, patients with non-CNS infections who had overwhelming or disseminated disease had a high mortality rate when treated with sulphonamides alone. However, currently available therapeutic alternatives are scarce and only comprise amoxicillin/clavulanate, carbapenems (imipenem, meropenem, doripenem, and ertapenem) and amikacin. Resistance to previously administered drugs, the toxicity and intolerance of the antimicrobials and even therapeutic failure necessitate the search for alternative agents. Results from *in vitro* studies suggest that nemonoxacin (TG-873870), a non-fluorinated quinolone, linezolid and tigecycline show promise as alternatives for the treatment of nocardiosis. Further clinical trials are needed to clarify their role in the treatment of these infections.

Novel influenza A (H1N1)

O501 Efficacy and safety of oseltamivir-zanamivir combination compared to each monotherapy for seasonal influenza: a randomized, double-blinded, placebo-controlled trial

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Objective: Evaluation of oseltamir-zanamivir combination efficacy and tolerance is of interest given the (H1N1) 2009 virus pandemic.
Methods: A randomized, placebo controlled, double blind study was conducted during the 2009 seasonal Influenza epidemic to evaluate oseltamivir-zanamivir combination. Adults with influenza like illness for less than 36 hours and a positive Influenza A rapid test diagnosis were randomized to oseltamivir 75 mg orally twice daily plus zanamivir 10 mg by inhalation twice daily (OZ), oseltamivir plus inhaled placebo (O) or zanamivir plus oral placebo (Z). Virological success was defined by a nasal viral load (RT-PCR) J2 <200 copies genome equivalents (cgeq) / ml. Clinical response was assessed by time to allevation as symptoms.
Results: Overall 541 patients were included (OZ, n = 173; O, n = 192; Z, n = 176). In the intention-to-treat analysis conducted in the 447 patients with confirmed Influenza A, the virological response was 46%, 59%, and 34% in OZ, O and Z arms ($p = 0.025$, $p = 0.028$ for OZ/O and OZ/Z comparisons). Mean viral load decrease D0-D2 was 2.14, 2.49, and 1.68 log10 cgeq /μL ($p = 0.060$, $p = 0.016$ for OZ/O and OZ/Z comparisons). The time of disappearance of symptoms were 4, 3, 4 days ($p = 0.030$, $p = 0.77$ for OZ/O and OZ / Z) The combination was well tolerated.
Conclusions: Monotherapy of oseltamivir remains the first line antiviral strategy for the ongoing Influenza pandemic. The lesser effect of the oseltamivir-zanamivir combination should be further investigated.

O502 Effectiveness and safety of neuraminidase inhibitors in reducing influenza complications: a meta-analysis of randomized controlled trials

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Objective: Several studies provide evidence that neuraminidase inhibitors can reduce the duration of influenza symptoms. Yet, data regarding their effectiveness in reducing influenza complications are scarce.
Methods: We evaluated the effectiveness of neuraminidase inhibitors in reducing influenza complications and mortality of patients with seasonal influenza, by performing a meta-analysis of randomized controlled trials (RCTs) retrieved from PubMed, Cochrane and Scopus databases, comparing neuraminidase inhibitors with placebo.
Results: Eleven RCTs (10 double-blinded. 1 with an open-label design) were included; 8 involved adults and adolescents. In total, 5315 patients were included; 3491 (65.7%) with confirmed infection. Total influenza-related complications occurred significantly less likely in otherwise healthy patients with confirmed infection treated with antivirals versus placebo [7 RCTs, 2621 patients, OR (odds ratio)=0.71, 95% confidence interval (CI)=0.58–0.87]. This finding was more pronounced in high-risk patients (4 RCTs, 475 patients, OR = 0.26, 95% CI=0.15–0.47), compared with otherwise healthy patients ($p < 0.01$). In the comparisons regarding other respiratory complications, a trend in favour of antivirals was observed. Regarding acute otitis media specifically, a significant difference was observed. Significantly fewer antibiotics were also administered in patients with confirmed infection treated with antivirals versus placebo (6 RCTs, 1921 patients, OR = 0.77, 95% CI=0.62–0.96). No differences were found in the comparisons regarding the safety outcomes of our meta-analysis. Mortality data were scarcely reported. No deaths were reported in the respective trials.
Conclusions: Neuraminidase inhibitors appear to be effective in reducing total influenza-related complications both in otherwise healthy with confirmed infection and high-risk patients. They also reduce additional antibiotic consumption.

O503 Clinical characteristics of influenza A H1N1–2009 outbreak at a public university hospital in the north-eastern region of Rio Grande do Sul, Brazil

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Objectives: In July 2009, a respiratory illness outbreak caused by influenza A virus (H1N1–2009) was identified in Caxias do Sul, Brazil. The aim of this work is to describe the clinical and epidemiologic characteristics of hospitalized patients at a public university hospital for viral pneumonia who had laboratory confirmed H1N1–2009 infection.
Methods: Retrospective medical chart reviews on the hospitalized patients between July and August 2009. H1N1–2009 infection was confirmed in specimens with the use of a real-time reverse-transcriptase–polymerase-chain-reaction assay.
Results: From July 1 through August 30, 2009, a total of 40 cases of confirmed H1N1–2009 pneumonia were identified among 124 patients hospitalized for acute respiratory illness at the Caxias do Sul General Hospital. More than half of the 40 patients were between 20 and 49 years of age, and only 10 had preexisting medical conditions. All patients had fever, cough, dyspnea or respiratory distress, increased serum lactate dehydrogenase levels and lymphopenia. Thirty-seven patients required mechanical ventilation, and five died.
Conclusions: In 2009, our region had the coldest winter season in many years. Influenza infections are common cause of respiratory diseases in our city, but on this year there was an increase of visits to doctors for influenza-like-illness and also flu-related hospitalizations. Comparisons with seasonal influenza suggest that pandemic influenza A (H1N1–2009) disproportionately affects younger ages and causes generally mild disease, but during the outbreak we noticed that H1N1–2009 infection had caused severe pneumonia and death in many previously young to middle-aged healthy persons.

O504 Hospitalized cancer patients with severe infections due to the novel influenza A (H1N1) in Brazil

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Background: The novel influenza A (H1N1) pandemia exposes immunocompromised cancer patients under chemotherapy to an increased risk of mortality.
Objective: To describe clinical characteristics, treatment, and outcome of cancer patients with 2009 Influenza A (H1N1) infection.

Methods: A descriptive study of hospitalized cancer patients at an Oncology Cancer Center in Brazil between July 8 and September 1, 2009 who were tested positive for the 2009 H1N1 virus with the use of a RT-PCR assay and/or a direct fluorescence antibody staining.
Results: As of 1 September, 24 cases were tested positive for influenza virus infection. Median age was 14.5 years (2–69 years). Underlying cancer diagnoses were: acute leukemia, 7 (29%); solid tumors, 6 (25%); lymphoma, 6 (25%); multiple myeloma, 3 (12%) and chronic leukemia, 2 (8%). All patients had fever and acute respiratory symptoms. Previous chemotherapy (79%), neutropenia (50%) and history of steroids therapy (37%) were commonly present. No patients had laboratory-documented bacterial infections at the illness onset, however all received antibacterial agents at presentation. Seventeen patients developed bilateral pulmonary infiltrates and severe hypoxemia was present in 15 cases (88.2%). Of these, 11 underwent mechanical ventilation. Twenty-two patients were treated with oseltamivir and, in 37.5% of them a double dose was prescribed. Median time from admission to antiviral therapy start was 5 days (range: 0–20 days). Antiviral therapy was started within 48 h of symptoms in only 10 patients (22%). Main reasons for therapy delay were the lack of specificity symptoms in neutropenic patients and the initial difficulty to obtain antiviral drugs. Five patients died (21%), and two of them never received oseltamivir. In the other 3 cases, the therapy was only started 8, 9 and 15 days after hospital admission.
Conclusions: This study presents a series of cancer patients with H1N1 virus infection with an elevated mortality. We observed a high number of cases in young patients. Considering the clinical data, a nosocomial transmission cannot be ruled-out. Our data shows a possible association of mortality with a lack or a delay of antiviral therapy initiation. A faster availability of antiviral medication for therapy and prophylaxis and vaccination programs should be reinforced among cancer patients, close contacts and healthcare workers.

O505 Epidemiology of severe paediatric patients with novel influenza A (H1N1) in Korea

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Objectives: Since the first outbreak of novel influenza A (H1N1) in May 2009, the virus has been spread throughout local communities. More than 4,000 diagnosed cases are being reported daily as of November 2009. One of the major infection routes is the educational institutions, so children and teenagers have very high risk of viral exposure. Recently, mass outbreaks were reported from 870 schools within one week. Korea Centers for Disease Control and Prevention (KCDC) is operating nationwide monitoring system for severe hospitalization cases. The objective of this study is to highlight demographics, infection risk factors and clinical courses.
Methods: Novel influenza A (H1N1) patients who were hospitalized in intensive care unit or had pneumonia in needs of intubation were categorized as severe pediatric patients. Between June and October, total of 22 cases under the age of 18 were identified as severe patients. After the medical chart review, we had an interview with the doctor in charge. All the patients were laboratory-confirmed novel influenza A (H1N1) virus infection by means of real-time polymerase chain reaction. Based on the Advisory Committee on Immunization Practices, the patients with high-risk medical conditions were defined as having higher risk for influenza complications.
Result: Among the reported 22 severe cases, 15 were male and 7 were female. Ages ranged from 2 months to 18 years old (median 7, standard deviation 5.4). Fourteen patients (63%) had high-risk medical conditions such as 1) age less than 59 months (6 cases), 2) chronic respiratory disease (3 asthma cases), 3) neuro-developmental disorder (3 cases), 4) congenital heart disease (1 case) and 5) leukemia (1 case). Total of 7 patients have expired. Patients took anti-viral agent (Tamiflu®) average 2 days after onset. Thirteen patients received ventilator care, 7 did not and 2 were unsure. Viral pneumonia was the most common complication (17 cases, 77%) and 3 patients exacerbated into acute respiratory distress syndrome. Initial symptoms were fever and cough (18 cases, 81% each). There were 11 leukocytosis, 3 leucopenia and 3 thrombocytopenia cases on complete blood count.
Conclusions: Half of patients with high-risk medical conditions have expired. Considering current situations, we need to maintain high-risk medical conditions category and to have continuous tracking for severe pediatric patients with novel influenza A (H1N1).

Table 1. Characteristics or status of Korean pediatric patients with influenza A(H1N1), June–October 2009

Characteristics/Status	No. of patients (N = 22)	Percentage (%)
Age group		
0–23 months	1	4.5
24–59 months	5	22.7
5–18 years	16	72.7
Sex		
Male	15	68.1
Female	7	31.9
High-risk medical conditions		
Ages under 59 months	6	27.2
Neurodevelopmental condition	3	13.6
Chronic pulmonary condition	3	13.6
Congenital heart disease	1	4.5
Immunosuppression	1	4.5
Antiviral tratment (tamiflu®)		
Unsure	2	9.0
⩽2 days after illness onset	15	68.1
>2 days after illness onset	5	22.7

Nosocomial pneumonia

O506 Ventilator-associated pneumonia rates in 12 intensive care units between 1995 and 2006, Lyon, France

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Objective: The aim of this study was to describe trends of ventilator acquired pneumonia (VAP) incidence in 12 intensive care units (ICU) by taking into account of individual major risk factors of VAP.
Methods: A prospective surveillance in 12 ICUs participating in the national nosocomial infection surveillance network in South-East France between 1995 and 2006 was done. The VAP was defined base on clinical, radiological and bacteriological findings, according to the national protocol. Yearly incidences of VAP were described. The risk of VAP by time was modelled using a Cox proportional hazard model with the year of admission in ICU as the main exposure. The tested covariates were age, gender, and SAPSII.

Table 1. Characteristics, incidences and risks of ventilator associated pneumonia in 12 intensive care units included in a surveillance program network, 1995–2006, Lyon, France

Year of participation	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Total
No. of patients	250	506	524	726	1678	1135	1191	1669	1552	1451	1539	1622	13843
No. of ICUs	2	3	3	4	10	9	9	10	9	10	9	9	87
No. of VAPs*	48	52	39	75	198	166	182	277	152	239	249	259	1936
Age (years), Mean (SD)	60 (17)	53 (20)	52 (21)	55 (20)	56 (19)	56 (20)	56 (20)	56 (21)	57 (18)	57 (18)	57 (18)	58 (17)	56 (19)
VAP incidence per 1000 patient-days	23.3	10.2	7.5	11.4	16.1	15.1	17.6	19.6	13.10	21.3	20.7	21.3	14.7
SAPSII*, Mean (SD)	35 (17)	40 (15)	40 (16)	43 (17)	40 (18)	41 (18)	41 (20)	37 (18)	46 (18)	47 (18)	47 (18)	48 (19)	43 (19)
Adjusted HR* (95% CI)[a]	1.00	1.09 (0.8-1.4)	0.47 (0.3-0.6)	0.34 (0.2-0.5)	0.53 (0.4-0.7)	0.75 (0.6-0.9)	0.70 (0.6-0.9)	0.87 (0.7-1.0)	0.95 (0.8-1.1)	0.62 (0.5-0.7)	0.97 (0.8-1.1)	0.96 (0.8-1.1)	

*SAPS II: Simplified acute physiology score II; VAP: ventilator acquired pneumonia, HR: hazard ratio adjusted on age, sex and SAPS II.
[a]Comparisons with year 1995, CI: confidence interval, SD: Standard deviation.

Results: Overall 13,843 patients were included, counting for 131,585 patient-days. A total of 1,936 VAP were observed. The mean incidence was 16.4/1 000 patient-days. The mean age was 56 years (Standard deviation = 32), and the mean SAPSII was 43 (Sd =19). Variations over time were observed for VAP incidence ($P < 0.001$), age ($P < 0.001$), and

SAPSII ($P < 0.001$), significant by the Pearson khi square test. After multivariate analysis, VAP risk per year decreased between years 1996 to 1998, increased continuously until 2001 and stabilized between 2002 and 2006, compared to 1995 (global Hazard Ratio=0.96; 95% CI [0.81–1.14]).
Conclusion: Despite of an increase of risk factors as age and SAPSII scores of ICU patients by time, we observed that the adjusted VAP risk between 2002 and 2006 were stable. These results should be a consequence of the implementation of specific prevention measures related to ICUs practitioners.

O507 Attributable mortality of ventilator-associated pneumonia: a meta-analysis

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Objective: To investigate whether ventilator-associated pneumonia (VAP) is a true cause of mortality in the intensive care unit (ICU) setting.
Methods: We performed a meta-analysis of available data obtained through search of PubMed and relevant bibliographies without time restrictions. A conservative DerSimonian-Laird random effects model was employed to calculate pooled odds ratios (OR) and 95% confidence intervals (CIs).
Results: Out of 968 retrieved reports, 44 papers fulfilled our inclusion criteria. Presence, as opposed to absence, of VAP was associated with higher mortality in the ICU setting (OR: 1.96, 95% CI: [1.26, 3.04]). This result persisted when matched case control studies (OR 1.73, 95% CI: [1.23, 2.45]) or studies in which VAP was microbiologically confirmed in all patients (OR: 2.20, 95% CI: [1.01, 4.81]) were evaluated separately. VAP was still associated with higher mortality when the impact of immunosuppresion was controlled (OR: 1.74, 95% CI: [0.95, 3.16]); a finding that did not reach statistical significance. Though, presence of VAP was not associated with higher mortality in the subgroup analysis of studies including patients who received appropriate initial antimicrobial treatment (OR 1.64 [0.68, 3.96]).
Conclusion: Presence, compared to absence, of VAP seems to be associated with higher mortality in critically ill patients. Appropriateness of initial antimicrobial treatment in such patients may moderate this association.

O508 Impact of antiseptic based oral care on rates of ventilator-associated pneumonia in intensive care unit patients: a meta-analysis

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Objective: Ventilator-associated pneumonia (VAP) is the most frequent nosocomial infection in the intensive care unit. Reducing inoculums of oral pathogenic microorganisms by adequate oral care could prevent VAP.
Methods: A systematic review of the literature concerning oral decontamination with chlorhexidine or povidone-iodine on intubated patients, and subsequent meta-analysis evaluating the effects of oral decontamination on the incidence of VAP was performed. Relevant articles were searched for in electronic databases as PubMed, CINAHL, Web of Science and CENTRAL and supplemented by manual searches of reference lists. Only randomized controlled trials evaluating the effect of oral care with use of chlorhexidine or povidone-iodine vs. oral care without use of an antiseptic on the incidence of VAP in adult intubated patients were included. Data were extracted as dichotomous variables. Data analysis was performed using RevMan 5.0. Statistical analysis was conducted according the Mantel-Haenszel model to obtain the relative risk (RR) and 95% confidence interval (CI). Heterogeneity was assessed using the Chi-2 test.
Results: Eleven studies were included in the analysis (n patients = 1971) of which 9 that investigated the effect of oral care with use of chlorhexidine (n patients = 1862) and 2 that assessed the effect of oral care with use of povidone-iodine (n patients = 109). The use of an antisepticum resulted in a significant reduction of the incidence of VAP with a RR of 0.63 (95% CI 0.50–0.81; $p = 0.0002$). These results are valid for chlorhexidine (RR 0.68; 95% CI 0.53–0.88; $p = 0.004$) and povidone-iodine (RR 0.38; 95% CI 0.19–0.75; $p = 0.005$). Among studies important differences exists concerning concentrations of the antiseptic used, frequency of oral care, and study methodology and diagnostic criteria for VAP. Clinical heterogeneity was confirmed statistically and was moderate ($\chi^2 = 43\%$; $p = 0.08$) for the trials using chlorhexidine and high ($\chi^2 = 66\%$; $p = 0.09$) for those assessing povidone-iodine. Subgroup analyses revealed most beneficial effects with concentrations of 0.12% and 2% chlorhexidine and 10% povidone-iodine, and in a population of cardiac surgery patients.
Conclusions: This analysis shows that oral decontamination with an antiseptic reduces the incidence of VAP significantly. Both chlorhexidine and povidone-iodine show this effect. However, further research is needed to make recommendations about the concentration and frequency of application.

O509 Efficacy of panobacumab, an IgM monoclonal antibody, in hospital-acquired pneumonia caused by *Pseudomonas aeruginosa*

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Objectives: Despite adequate antibiotic therapy Hospital Acquired Pneumonia (HAP) and Ventilator Associated Pneumonia (VAP) caused by *P. aeruginosa* are of the most common and deadliest nosocomial infections. Panobacumab, a fully human IgM/kappa monoclonal antibody targeting *P. aeruginosa* serotype O11, was evaluated as a new therapeutic modality for treatment of VAP/HAP.
Methods: Patients with HAP caused by *P. aeruginosa* O11 were enrolled in a Phase IIa open trial to be treated with Panobacumab 1.2 mg/kg (days 1, 4, 7) in addition to standard antibiotic therapy.
Results: 17 patients, (23–83 years) with VAP (n = 14) or HAP (n = 3) were treated with Panobacumab. Thirteen patients (completers) received 3 infusions and 4 patients (non-completers) received 1 infusion. The study drug was safe and well tolerated. Panobacumab revealed a pharmacokinetic profile similar to a native IgM. Panobacumab could be detected in BAL samples collected after treatment indicating the antibody to reach the inflamed lung tissue. Clinically the initial mean CPIS and APACHEII score were 8.53 (7–11) and 18.2 (6–33) with an expected mortality of 29.6%. The overall observed mortality within 30 days after starting treatment was 17.6%. Despite an APACHEII score of 18.5 and expected mortality of 30.6% all completers survived within the first 30 days while non-completers showed a survival rate of 25%. Resolution of pneumonia was achieved by 9 out of 13 completers in 9.9±4.3 days (mean±SD) including two patients which were initially inadequately treated. An early administration of the antibody seems to correlate positively with the resolution of pneumonia. The mean Cmax after the third dose of Panobacumab was higher in patients with resolution than in those with continuation of pneumonia (34.8 versus 28.5 mg/L) as well as in those with eradication of *P. aeruginosa* versus those with continuation (37.3 versus 27.7 mg/L).
Conclusions: A survival of 100% was observed in patients that completed the full treatment cycle with 3 doses of Panobacumab indicating efficacy of the antibody treatment. Early administration of Panobacumab and high Cmax levels seem to correlate positively with the resolution of pneumonia and eradication of *P. aeruginosa*. The current data is promising and warrant further trials with Panobacumab.

O510 Tigecycline in the treatment of multidrug-resistant *Acinetobacter baumannii* pneumonia

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Objectives: Nosocomial infections by multidrug-resistant (MDR) *A. baumannii* are important causes of mortality in intensive care units

(ICU). The treatment choices are limited. The aim of this study was to evaluate the efficacy of tigecyline in MDR *A. baumannii* pneumonia.
Method: This study was performed at a tertiary-care educational university hospital with an active respiratory diseases ward with 100 beds, 8 of which are in ICU. We retrospectively evaluated the outcome of adult (>18 years old) patients with culture proven MDR *A. baumannii* pneumoniae treated with tigecycline between January 2009 and November 2009. Demographic, clinical and laboratory data, predisposing factors, as well as information on response to treatment and outcome were obtained from each patient's hospital records.
Results: There were a total of 34 cases (18 male, mean age 66.79±14.84 years) fulfilling our inclusion criteria. On the admission 17 cases were diagnosed as community-acquired pneumonia and 12 were diagnosed as chronic obstructive lung disease. 27 cases (79.4%) had comorbidities and the most common comorbidity was atherosclerotic heart disease (8 cases) followed by diabetes mellitus (5 cases), cerebrovascular disease (5 cases), respectively. When *A. baumannii* was isolated from respiratory samples 19 were considered as ventilator-associated pneumonia and 15 were considered as hospital-acquired pneumonia. All isolates were sensitive to tigecycline, whereas all were resistant to piperacillin/tazobactam and ceftazidime, 74%, 56%, 41% and 24% were resistant to imipenem, cefoperazone/sulbactam, amikacin and netilmicin, respectively. Tigecycline was used for a mean duration of 9.6±5.2 days. Microbiologic eradication (on day 3 and 7) was observed in 21 cases (61.8%). Tigecycline was combined with cefoperazone/sulbactam in 12 cases, with netilmicin in 6 cases and with amikacin in 3 cases. Nine of 21 cases with microbiologic eradication were lost whereas mortality was 100% in the other 13 cases ($p = 0.0006$). Mortality and microbiologic eradication rates were not different in combination vs monotherapy ($p > 0.05$). Mortal cases were elder than the survivors (71.1±12.6 vs 58.9±15.9, $p = 0.02$). Toxicity developed only in one case as liver toxicity.
Conclusion: Our findings show that microbiologic eradication rate of tigecycline in MDR *A. baumannii* is not very low. However this microbiologic eradication rate did not result in good clinical results in terms of mortality probably due to comorbidities.

Daptomycin – clinical experience

O511 Daptomycin for the treatment of infective endocarditis: results from European Cubicin® Outcomes Registry and Experience (EU-CORE)

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Objective: Randomized controlled trials are scarce in endocarditis and might not be representative for the general patient population. The aim of this registry was to describe the clinical experience with Daptomycin (DAP) for the treatment of infective endocarditis (IE) in Europe. DAP is approved in Europe for the treatment of right-sided endocarditis due to *S. aureus* at a dose of 6 mg/kg once daily.
Methods: In this multi-centre, retrospective, non-interventional registry, the outcome data were collected in patients (pts) who were diagnosed with native or prosthetic valve IE and treated with at least one dose of DAP. Clinical outcomes were assessed at the end of DAP treatment by the investigators using standard definitions (cured, improved, failure, non-evaluable). Success was defined as the sum of cured or improved outcome rates.
Results: Of the total 2581 pts in the EU-CORE registry from Jan 2006 to Aug 2009, 276 pts (68% male and 53% ⩾65 years of age) had IE. A total of 117 (42%) pts received DAP in an ICU, congestive heart failure was present in 31 (11%) pts and cardiac arrhythmias in 59 (21%) pts. Right sided endocarditis was observed in 66 (24%) pts, left sided endocarditis in 191 (69%) pts, and right plus left sided endocarditis in 19 (7%) pts, respectively. The most common primary pathogen was *S. aureus* (n = 73, 26%), of which 25 were reported as MRSA. 180 (66%) pts received concomitant antibiotic therapy, most commonly aminoglycosides (71, 26%) or carbapenems (46, 17%). The majority of pts received DAP doses of 6 mg/kg (62%) or higher (21%). The clinical outcome per IE type were:
Right sided endocarditis, success 92%, failure 5%, non-evaluable (NE) 3%;
Left sided endocarditis, success 76%, failure 9%, NE 14%;
Right plus left sided endocarditis, success 89%, NE 11%.
The proportion of pts with low Creatinine clearance (<30 mL/min) improved from 17% (46/276) initially to 13% (35/276) at the end of DAP treatment. Serious AEs were reported in 34 (12%) pts and 20 (7%) pts discontinued the study drug due to AEs.
Conclusions: Daptomycin appears effective and well tolerated against a variety of clinical presentations of infective endocarditis. Success rates and adverse event profile, including renal safety were comparable to those observed in the pivotal trials. Further clinical studies on infective endocarditis e.g. on the impact of DAP at higher doses might be warranted.

O512 High-dose daptomycin for infective endocarditis

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Objective: Infective endocarditis (IE) is a serious infection associated with high morbidity and mortality. IE due to methicillin-resistant *Staphylococcus aureus* (MRSA) and enterococci including vancomycin-resistant strains (VRE) has been associated with high failure rates. Daptomycin (DAP) has activity against both MRSA and VRE strains. We evaluated the safety and efficacy of high-dose (HD) DAP (⩾8 mg/kg/day) for MRSA and VRE IE.
Methods: Consecutive patients treated with HD DAP for ⩾72 h, excluding dialysis, were retrospectively reviewed at 4 academic medical centres. This subset analysis included all patients with IE defined by modified Duke criteria. Charts were reviewed for demographics, comorbidities, antimicrobial therapy, microbiologic cultures, clinical outcomes, and adverse events.

Table 1.

	Right-sided endocarditis (RIE) (n = 23) Median (range) or n (%)	Left-sided endocarditis (LIE) (n = 20) Median (range) or n (%)
Primary Organism		
MRSA	19 (82.6%)	12 (60.0%)
VRE	1 (4.3%)	4 (20.0%)
No organism isolated	3 (13.0%)	4 (20.0%)
Clinical and Microbiological Outcomes		
Duration of bacteraemia (days)		
MRSA (n = 31)	6 (1–30)	12 (6–18)
VRE (n = 5)	1	12 (1–12)
Duration of fever (days)	4.5 (1–21)	8 (0–21)
Duration of leukocytosis (days)	10 (1–44)	11.5 (1–44)
Duration in ICU (days)	8.5 (0–13)	12 (1–79)
Duration of HD DAP (days)	8 (3–27)	12.5 (3–48)
Length of stay (days)	17 (8–57)	25 (10–91)
Median dose of HD DAP (mg/kg/day)	9.7 (8.0–11.4)	9.9 (8.0–12.1)
Clinical success		
MRSA (n = 31)	13 (81.3%)	9 (90.0%)
VRE (n = 5)	1 (100%)	3 (75.0%)
Clinical failure		
MRSA (n = 31)	3 (18.8%)	1 (10.0%)
VRE (n = 5)	0 (0%)	1 (25.0%)
Microbiological eradication	19 (86.4%)	19 (95.0%)
Organism persisted	3 (13.6%)	1 (5.0%)
Safety		
EOT CPK <150 IU/L	20 (87.0%)	17 (85.0%)

Results: 45 patients were identified. Baseline characteristics: Median age 53 years (range 24–93), APACHE II 7 (0–28), 57.8% hospitalization ⩽1 year, 22.2% MRSA infection ⩽1 year, 57.8% injection drug use, 28.9% diabetes, 22.2% renal disease. 23 (51.1%) patients had right-sided IE (RIE), 20 (44.4%) patients had left-sided IE (LIE), and 2 (4.4%) patients had both RIE and LIE. Characteristics and outcomes of patients with RIE or LIE are presented in Table 1. The median days of bacteraemia, as determined from clinical cultures, for MRSA RIE and LIE were 6 days (1–30) and 12 days (6–18), respectively. Overall clinical cure and microbiological eradication was 76.1% and 86.4%, respectively. The median dose of HD DAP for RIE vs. LIE were 9.7 mg/kg/day (8.0–11.4) vs. 9.9 mg/kg/day (8.0–12.1), respectively. Safety: 87% patients had

end-of-therapy (EOT) creatinine phosphokinase (CPK) levels <150 IU/L (15–452). No patients were discontinued from therapy due to myopathy or any other adverse events.
Conclusion: Efficacy and safety of HD DAP were favourable in a cohort of pts with IE. Further studies with a larger cohort are warranted.

O513 Evaluation of safety and tolerability of daptomycin in the treatment of osteomyelitis: results from a European Registry

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Objectives: Osteomyelitis (OM) is a complicated and diverse disease and long term efficacy and safety of an antibiotic is an important determinant in selecting the optimal therapy. This is the first report of a large series of OM cases treated with daptomycin (DAP) across many countries in Europe.
Methods: Data were collected as part of the European CUBICIN® Outcomes Registry and Experience (EU-CORE) program, a retrospective, non-interventional, observational, multicenter study designed to describe the clinical use of DAP. All patients (pts) who had a diagnosis of OM were selected from the database. Pts who were treated with at least one dose of DAP and for whom any safety parameter was assessed were included in safety population. Efficacy population was a subset of the safety population where clinical outcome (cure, improved, failure, or non-evaluable) was assessed by the investigators. Pts enrolled from Jan 2006 to Aug 2009 were included.
Results: Of 2581 pts enrolled 161 (58.4% male, 42% ⩾65 years) were diagnosed with OM. 90 (56%) cases of OM were non-prosthetic device related, 50 (31%) were permanent prosthetic device related, 21 (13%) were temporary prosthetic device related. For 103 pts (64%) primary pathogens have been reported. *S. aureus* (45/103) was the most frequent species, with MRSA (n = 19) as significant subset. Coagulase-negative Staphylococci (41/103) ranked second. Most pts received DAP doses ⩾6 mg/kg (76%). Median duration of DAP outpt therapy was 29 days (range: 4–82), and inpt therapy was 14 (range: 1–246). Overall clinical success (cured or improved) was seen in 73% (118/161) of pts and failure in 9% (14/161). 18% of pts were non-evaluable (29/161). In subgroup analyses, clinical success was observed in 72% (65/90) of pts without devices, 72% (36/50) of pts with permanent devices and 81% (17/21) of pts with temporary prosthetic devices. Serum creatine phosphokinase (CPK) values were reported in 68% (109/161) of pts during DAP therapy. In 86% of pts (94/109) CPK levels remained within normal range. Discontinuation of DAP due to treatment failure was reported for 2 pts (1%) only. Serious AEs were reported in 3% (5/161) of pts. AEs leading to study drug discontinuation were reported in 5% (8/161).
Conclusion: DAP at doses ⩾6 mg/kg once daily was the most frequent dose regimen used for osteomyelitis in Europe. DAP was well-tolerated also for longer treatment duration. These promising results warrant confirmation in additional studies.

O514 Daptomycin versus vancomycin for methicillin-resistant *Staphylococcus aureus* bacteraemia with reduced *in vitro* susceptibility to vancomycin

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Background: Poor outcomes in MRSA bacteremia have been associated with reduced *in vitro* susceptibility to vancomycin, yet still within the susceptible range. Data for alternative management in these cases is lacking, particularly with daptomycin (DAP).
Methods: We conducted a case–control study evaluating treatment with VAN or DAP for MRSA-B with a VAN MIC of 1.5 or 2 ug/mL. Patients were matched 2:1 by risk level of source, APACHE-II score at onset of infection and age. Respiratory infections were excluded. Failure was a composite of: 30-day mortality, microbiologic failure (positive cultures >10 d from index culture) and/or recurrence of MRSA-B within 30 days of end of tx.
Results: DAP treated patients (n 37) were compared to VAN treated patients (n 74). VAN MIC was 1.5 (46 v 85%) and 2.0 (54 v 15%), $p < 0.01$. Source of infection was similar between groups including endocarditis (27 v 24%), skin (38 v 32%), catheter (19 v 19%), device (8 v 11%) and other (8 v 14%). Age, comorbidities, APACHE II score (14 v 14) and requirement of ICU admission (24 vs 21%) was similar between groups, $p > 0.05$. Immunosuppression (24 v 7%) was more common in the DAP group, $p < 0.05$. Factors significantly associated with failure; ICU at onset of infection, nursing home residence, CV disease, DM, and ARF, $p < 0.05$. IVDA was significantly associated with cure, $p < 0.05$. Factors associated with 30 day mortality; Caucasian race, ICU at onset of infection, NH residence, ARF, $p < 0.05$. Hemodialysis was significantly associated with survival, $p < 0.05$. VAN MIC by Etest had no association with failure or mortality. There was a trend toward less failure (14 v 28%) and lower 30-day mortality (3 v 16%) in the DAP group, $p = 0.06$. Failure by source of infection was; endocarditis (10 v 39%), skin (14 v 17%), catheter (14 v 14%), device (33 v 75%) and other (0 v 20%). Mortality by source of infection was; endocarditis (0 v 28%), skin (7 v 8%), catheter (0 v 14%), device (0 v 13%) and other (0 v 20%).
Conclusion: Between groups, baseline comorbidities, risk level of source and APACHE-II scores were similar, although immunosuppresion and isolates with VAN MIC=2 were greater in the DAP group. This study shows that DAP may be associated with better outcome of infection as compared to VAN, and trended toward lower 30-day mortality, particularly in subjects with MRSA endocarditis.

	Daptomycin n 37	Vancomycin n 74	P
Age (Mean, SD)	50 (13)	51(15)	0.68
Male gender	65%	65%	1.00
Risk level of source			1.00
Low	19%	19%	
Intermediate	38%	38%	
High	43%	43%	
APACHE-II (mean, SD)	14 (7)	14 (7)	0.86
ICU at onset of infection	24%	21%	0.68
Cardiovascular disease	38%	37%	0.89
Diabetes mellitus	35%	39%	0.68
Acute renal failure	30%	23%	0.44
Hemodialysis	24%	24%	1.00
Liver disease	41%	28%	0.20
Malignancy	3%	10%	0.27
Immunosuppression	24%	7%	0.01
Nursing home resident	5%	14%	0.33
IVDA	41%	28%	0.20
Epidemiologic source			0.47
Community-associated	27%	34%	
Healthcare-associated	73%	66%	
Vancomycin MIC (μg/mL) Etest			<0.01
1.5	46%	85%	
2.0	54%	15%	

O515 Daptomycin against highly resistant *Enterococcus faecium* invasive infections

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Objective: Treatment recommendations for invasive enterococcal infections advocate synergistic combinations such as ampicillin plus gentamicin. In case of resistance to one or even both of the drugs, vancomycin therapy is proposed, albeit it is poorly bactericidal. The Swiss surveillance program reports resistance of *Enterococcus faecium* to aminopenicillin and high-level gentamicin to be as high as 82% and

49%, respectively (www.anresis.ch). This leaves the clinician with a therapeutic conundrum since no established therapeutic regimen exists. Daptomycin is approved for complicated skin and soft tissue infections with Gram-positive bacteria and showed efficacy against *E. faecium* in the experimental endocarditis model.
Methods: We report a case series of 11 patients with severe *E. faecium* infections treated with daptomycin at the University Hospital Basel between 2007 and 2009. All strains were resistant to ampicillin (MIC > 8 mg/l), but susceptible to vancomycin. 7/11 strains were also highly resistant to gentamicin (MIC > 500 mg/l).
Results: All patients were treated with multiple broad-spectrum antibiotics prior to isolation of *E. faecium* and had severe underlying diseases: Five had haematological malignancies, two had repeated episodes of cholangiosepsis, two suffered from severe atherosclerosis after multiple vascular surgical procedures, one had undergone liver transplantation and one had colon cancer. Foci were mainly blood stream infections or cholangitis. The reasons for daptomycin treatment were renal failure (n = 3), vancomycin failure (n = 4) despite vancomycin susceptibility (MIC ⩽4 mg/l), outpatient parenteral therapy (n = 2) and uncontrolled infection (n = 2).
With one isolate from a patient with persistent bacteremia, *in vitro* kill-curves were performed, showing a bactericidal effect of daptomycin (loss of >3 log/CFU/24 h with 2 mg/l and 10 mg/l of the drug). In contrast, 10 mg/l of vancomycin was bacteriostatic. Daptomycin was used in a dose of 6 mg/kg/d. 7 patients recovered, 4 patients died. Death was related to uncontrollable infection in only one case, the other deaths were attributable to the underlying diseases.
Conclusion: Our case series suggests that salvage therapy with daptomycin was likely to be efficient in 7/11 patients with refractory invasive infections due to multi-resistant *E. faecium.* Thus, daptomycin might be a safe option in such cases.

PK/PD approaches in Gram-negative infections

O516 Pharmacokinetic evaluation of intravenous colistin following two different dose regimens in multidrug-resistant infections

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Objectives: Infections caused by multidrug-resistant Gram-negative bacteria are a growing clinical problem and are associated with significant morbidity and mortality. We evaluated colistin concentrations at steady-state in plasma samples and bronchoalveolar lavage (BAL) of critically ill patients admitted to our ICU.
Methods: Nineteen patients (16M, 3F) aged 20–70 years and affected with ventilator-associated pneumonia were enrolled. Six patients received 1 million IU of colistin methanesulphonate (CMS) intravenously every 6 hours; thirteen patients received 2 million IU every 8 hours for at least 2 days. Blood samples were collected from each patient at baseline (predose) and at time intervals after the end of CMS infusion. BAL was performed in all patients at 2 hours post-infusion. Colistin plasma and bronchoalveolar concentrations were measured using a selective and sensitive high performance liquid chromatography assay with fluorescence detector. Pharmacokinetic parameters were determined by non-compartmental analysis using Innaphase Kinetica™ 4.0 software.
Results: Patients receiving 1M IU/6 h had mean [±SD] Cmax and Cmin plasma concentrations at the steady-state of 1.57 [±0.57] and 1.10 [±0.43] μg/ml, respectively. Mean [±SD] AUC(0–6 h), t1/2 and Vd were 6.40 [±2.32] μg·h/ml, 8.36 [±4.94] h, and 220.46 [±76.61] L/h, respectively. Patients receiving 2M IU/8 h had mean [±SD] plasma Cmax and Cmin levels at steady-state of 2.21 [±1.08] and 0.98 [±0.70] μg/ml, respectively. Mean [±SD] AUC(0–8 h), t1/2 and Vd were 11.54 [±6.20] μg·h/ml, 5.87 [±2.56] h, and 143.24 [±116.47] L/h, respectively. Cmax/MIC and AUC(0–24 h) /MIC ratios (MIC = 2 μg/ml) were 0.79 [±0.28] and 12.79 [±4.65], 1.1 [±0.54] and 17.30 [±9.30], after administration of 1M IU and 2M IU, respectively. Colistin was undetectable in BAL under both regimens. A complete eradication of bacteria was observed in 12/13 of our patients with the 2M IU/8 h dosing schedule.
Conclusions: In critically ill patients, the 2M IU/8 h dose regimen provided higher Cmax and AUC than the 1M IU/6 h schedule: this may be a therapeutic advantage because the AUC/MIC and Cmax/MIC ratios are strongly associated with efficacy. Further studies are needed to provide important clinical answers, from individualising treatment to optimising dosage and reducing adverse effects.

O517 Pharmacokinetic/pharmacodynamic modelling of polymyxin B, rifampicin and tigecycline against pandrug-resistant *Acinetobacter baumannii* in an *in vitro* model

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Objective: Outbreaks of pandrug-resistant (PDR) *Acinetobacter baumannii* (AB) have emerged in Singapore. Combination therapy is often the remaining viable option until new antibiotics are available. While polymyxin B (P) may remain a viable treatment option, heteroresistance has become a major problem. We evaluate the efficacy of P, rifampicin (R) and tigecycline (T) combined against PDR AB isolated from our local hospitals.
Methods: PDR AB isolates from all public hospitals in Singapore were collected from 2006–07. MICs were determined according to a modified CLSI broth-dilution method. Time-kill studies (TKS) were then performed with the maximum, clinically achievable, unbound concentration (mg/L) of P (2), R (2) and T (2) alone and in combination against the PDR AB isolates. A hollow-fiber infection model (HFIM) was used to validate our quantitative assessment of combined killing against 2 isolates (selected based on the unique genotype that represents the PDR AB population). Resistance selection of the 2 isolates against P alone in the HFIM were quantified using drug-free and selective (P at 3x MIC) media.
Results: Among 361 non-repeat AB isolates screened, 29 PDR AB isolates found were susceptible to P (MICs 1–2 mg/L) and resistant to all antibiotic classes whereas R MICs ranged from 2–16 mg/L. In TKS, P, R and T alone was bacteriostatic with regrowth by 24 h in all isolates. P+R, P+T and R+T achieved >99% kill from baseline in 15/29, 14/29 and 8/29 isolates with no regrowth at 24 h. These assessments were consistent with observation in HFIM studies where we observe bacterial killing up to 120 h with P+R. Pharmacokinetic validation of the HFIM studies were satisfactory. Minimal killing of the 2 isolates was seen when exposed to P alone. Regrowth was seen at 24 h due to selective amplication of resistant sub-population(s) on P supplemented plates. Repeat MIC testing of the resistant isolates confirms P resistance (MICs 32–128 mg/L).
Conclusions: We have shown that our PDR AB has the propensity to exhibit heteroresistance and combination therapy with P is needed. P+R may be a potential antibiotic combination as therapy for PDR AB infections. The *in vivo* relevance of our results warrants further investigations.

O518 Pharmacodynamic evaluation of the intracellular activity of tobramycin, doripenem, levofloxacin, and colistin towards *Pseudomonas aeruginosa* after phagocytosis by human THP-1 macrophages

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Objectives: *P. aeruginosa* (Pa) is capable of invading epithelial and phagocytic cells (Mol. Biol. Cell 2005; 16: 2577–85), which may play an important role in the initiation and persistence of the infection process. As no data is available about antibiotic activity against intracellular P.a., we have developed a 24-h infection model using THP-1 cells, a human cell line known to be permissive for infection by several important human pathogens. This model has now been used to test for the activity of 4 antibiotics representative of 4 classes of drugs used in clinics to treat pseudomonal infections.
Methods: Phagocytosis of bacteria (opsonized with human serum) was allowed for 2 h (bacteria/macrophage ratio: 10), extracellular bacteria

were eliminated by washing and incubation for 60 min with gentamicin at 50 x MIC, and Infected cells ($5–7\times10^5$ CFU/mg cell prot.) incubated with antibiotics (0.01 to 100 × MIC). Activity against bacteria in broth (1×10^6 CFU/ml) was determined in parallel (same conc. span). Activity was expressed as change from the initial inoculum after 24 h, and the data used for fitting a concentration-response curve (Hill equation) and for calculation of the Emax and Cstatic pharmacodynamic parameters (AAC 2006; 50: 841–51).

Results: The table shows that while TOB and DOR were cidal in broth (Emax ≈ –3 log CFU), their intracellular maximal efficacy was markedly reduced (less negative Emax) towards intracell. Pa. LVX was also affected but to a lesser extent. CST, bacteriostatic against extracell. bacteria, also showed decreased intracellular efficacy. In addition, CST and TOB showed a 6 to 9 fold decrease of relative potency (higher Cstatic) when comparing intracell. and extracell. bacteria.

Conclusions: The model shows that, as for other intracell. bacteria, antibiotics are considerably less active and, for 2 classes, less potent, against intracellular forms compared to bacteria in broth, irrespective of their mode of action. This may contribute to the difficulty of eradicating *P. aeruginosa in vivo*.

Antibiotic	MIC[a]	Extracellular			Intracellular		
	(mg/L)	E_{max}[b]	C_{static}[c]	R^2	E_{max}[b]	C_{static}[c]	R^2
Tobramycin (TOB)	0.5	–2.85±0.31	0.86	0.92	–1.22±0.22	7.93	0.95
Doripenem (DOR)	0.5	–3.19±0.28	0.30	0.86	–0.21±0.2	0.59	0.61
Levofloxacin (LVX)	1–2	–3.17±0.36	0.15	0.69	–2.40±0.21	0.38	0.86
Colistin (CST)	2	–1.52±0.11	3.34	0.99	–0.20±0.17	18.84	0.93

[a] Determined by microdilution in MH broth; [b] maximum decrease in log CFU compared to initial inoculum for an infinitely high concentration in antibiotic; [c] concentration (in mg/L) resulting in no apparent bacterial growth (number of CFU identical to the original inoculum), as determined by graphical intrapolation.

O519 Population pharmacokinetics and pharmacokinetic–pharmacodynamic metrics for delafloxacin

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Objectives: To develop a population pharmacokinetic (PK) model for delafloxacin (DFX) and use that model to estimate pharmacokinetic–pharmacodynamic (PK-PD) indices in Phase 2 patients treated for complicated skin and skin structure infections (cSSSI).

Methods: Data from 3 Phase 1 studies (1 single dose, two multiple dose) and 1 Phase 2, cSSSI study were pooled to develop a population pharmacokinetic model. All doses were administered by IV infusion; doses ranged from 50 to 600 mg. The Phase 2 study employed doses of 300 or 450 mg BID. Intensive PK sampling was employed in all Phase 1 studies while Phase 2 patients contributed 4–5 samples at steady-state over one dosing interval. Population PK modeling was performed using Monte Carlo Parametric Expectation Maximization as implemented in S-ADAPT 1.5.6. Two and 3 compartment models were explored using linear and/or nonlinear elimination. Steady-state AUC and Cmax estimates were calculated using individual PK parameter estimates for Phase 2 patients and indexed to observed pathogen MICs to calculate PK-PD indices. Previous animal studies have indicated that free-drug AUC:MIC ratios (fAUC:MIC) of 9.3 and 14.3 are associated with net bacterial stasis and 1-log10 CFU reduction, respectively, for *Staphylococcus aureus*.

Results: The final analysis dataset included 103 subjects (86 from Phase 1, 17 from Phase 2) and 2273 plasma concentrations. A 2 compartment model with a mix of linear and nonlinear elimination provided the most robust fit to the data ($r^2 = 0.965$, observed=1.02*fitted – 0.032). All parameters were estimated with excellent precision; inter-individual variability in the parameters that define clearance (linear clearance, intrinsic clearance, and Michaelis-Menten constant) ranged from 24–70%. Twelve patients had requisite MIC data to calculate a PK-PD index, all fAUCss:MIC estimates were above 100 (range: 108–3754); all 12 patients were classified as clinical and microbiological cures. The median (min – max) fCmax:MIC ratio was 191 (18.8–565). Seven patients had MRSA as their primary pathogen; MIC values were universally low (0.004–0.06).

Conclusions: Development of a population PK model allowed for the estimation of drug exposure in a Phase 2 cSSSI study using relatively sparse PK sampling. Based on PK-PD indices from the Phase 2 study, 300 and 450 mg (BID) clinical doses were appropriate for the treatment of cSSSI. Results also support coverage of MIC values up to 1.0 mcg/mL.

O520 Pharmacokinetics and penetration of ciprofloxacin into bronchial secretions of critically ill patients with chronic obstructive pulmonary disease

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Objective: Ciprofloxacin (CIP) is one of the antibiotics of choice for the treatment of severe exacerbations of Chronic Obstructive Pulmonary Disease (COPD). Antibiotic penetration into the site of infection is critical for obtaining an optimal clinical outcome. Since in COPD patients the infection develops within the airway lumen, it is important to know the drug concentrations that are achieved in bronchial secretions (BS). The purpose of this study was to evaluate CIP's pharmacokinetic (PK) profile in plasma and penetration into BS of mechanically ventilated COPD patients, when administered at the currently recommended dose of 1200 mg per day.

Methods: Ninenteen critically ill COPD patients received a 1-hour infusion of 400 mg CIP q8 h. They all had a respiratory infection as well as risk factors for *Ps. aeruginosa* and they were intubated. Serial blood and BS samples were obtained at steady state. Concentrations were determined by a validated HPLC assay. Penetration ratio was calculated by dividing the 24 h area under the curve ($AUC_{0–24}$) of BS by the $AUC_{0–24}$ of plasma. The pharmacodynamic (PD) parameters for CIP were also calculated.

Results: Mean±SD values for volume of distribution, clearance, half-life and $AUC_{0\rightarrow24}$ were 174.31±85.42 L, 27.32±9.53 L/h, 5.5±2.34 h and 47.58±18.07, respectively. The mean peak (C_{max}) and trough levels in plasma were 5.32±1.76 and 1.05±0.59 mg/L respectively. In BS, a mean C_{max} of 3.11±1.27 mg/L was achieved in 3±1.03 hours and the penetration ratio was 1.17±0.61. Thirteen patients demonstrated penetration equal to or even more than 100%. The PD target of $AUC_{0\rightarrow24}$/MIC ⩾125 in plasma, that has been shown to be predictive of efficacy for Gram-negative infections, was attained in all patients and in the majority of them (74%) at MICs of 0.125 and 0.25 μg/ml respectively but in only 3 patients and in none at higher MICs (0.5 and 1 μg/ml). Slightly better results were obtained for the PD threshold of C_{max}/MIC ⩾ 10.

Conclusions: CIP exhibits excellent penetration into BS. There is wide interindividual variability in its PK parameters in critically ill COPD patients and inadequate PD exposure against bacteria with MICs ⩾0.5 μg/ml. Our data confirm the need for combination therapy against pathogens with high MICs such as *Ps. aeruginosa*, as well as the institution of therapeutic drug monitoring for individualizing antimicrobial dosing in order to optimize the efficacy of antibiotic therapy in the ICU.

Biofilm infections – diagnostic, prophylactic and therapeutic methods

S521 Diagnosis of biofilm infections

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The initial problem or challenge with all infections is to identify the infecting organisms and the focus of the infection. This is usually not a problem for acute infections since the bacteria are readily obtained by swapping or sampling the infected area. For chronic infections it is usually more problematic. An exception is cystic fibrosis (CF) in which the easy accessible purulent sputum coughed up by the patients on a regular basis harbor the bacteria. For the other chronic infections routine sampling has been either using a swab, a scrape or a biopsy, however all

might fail to sample the bacteria. In a chronic wound the swab will only collect bacteria on the surface not the bacteria embedded in the wound bed. On the other hand, since the bacteria are very heterogeneously distributed chances are that a biopsy fails to contain any bacteria. Also for implant and catheter related infection diagnosing the bacteria prove difficult. Five to 10 years ago the bacteria on these surfaces and biofilm in general were considered unculturable. Here the problem is surface adherence, the bacteria simply attach extremely well to the surface of the foreign bodies. It is not that they are unculturable, they have to be released from the surface, and vigorously vortexing or even mechanical scraping is not enough. The implant or catheter has to be treated with ultra sound (sonication) to release the bacteria.
The problems of diagnosing the bacteria in these chronic infections are far from solved. Today bacteria can be detected by: culturing, PCR, microscopy or diagnostic imaging. Each method has its advantages and limitations. For culturing, the problem is to collect the bacteria, either next to the surface, which is sampled, or from the catheter or implant. PCR will detect even tiny amounts of available DNA or RNA available in the sample. Additionally, just because a bacterium is present does not necessarily indicate that it contributes to the pathogenesis of the infection. Microscopy such as Gram-staining or fluorescence in situ hybridization (FISH) enables direct visualization of the infecting bacteria and the surrounding tissue and inflammatory cells. Again the bacteria need to be present in the collected sample, which means many biopsies need to be analyzed for a correct diagnostics of e.g. a wound. On the other hand if only a few bacteria are present they might be very hard and statistically impossible to observe using traditional staining such as Gram.

S523 Antibiotic therapy of biofilm-forming group A streptococcal infections

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Streptococcus pyogenes (group A streptococcus, GAS) is responsible for a diverse range of clinical manifestations, from mild skin/soft tissue infections and pharyngitis to more serious manifestations, such as bacteremia, cellulitis, puerperal sepsis, meningitis, pneumonia, and necrotizing fasciitis.
The drug of choice for streptococcal infections treatment still remains penicillin. In fact, the ability of penicillin and its related antibiotics (e.g., amoxicillin) to kill group A streptococci has not changed in more than 50 years and, to date, there has never been a report on a group A streptococcus resistant to this class of antibiotics. On the other hand, macrolide resistance has been showing an increasing trend, with resistance rates which vary considerably in different countries, reaching up to almost 30% in some part of Europe.
Effective treatment is of utmost importance, even for streptococcal pharyngitis, as it is primarily aimed at preventing non-suppurative and suppurative complications and decreasing infectivity.
Even if not frequently, *S. pyogenes* infections may fail to respond to antibiotic therapy leading to persistent throat carriage and recurrent infections. Such failures cannot always be explained with the occurrence of antibiotic resistance determinants. It was first suggested that erythromycin-resistant *S. pyogenes* may escape antimicrobial treatment and the host immune response through invasion of epithelial cells. Later, as GAS have recently been shown to be able to form biofilm, and being such character known to provide organisms with an improved antibiotic resistance besides supporting colonization and persistence, biofilm has been suggested as possibly responsible for unexplained treatment failures and recurrences due to susceptible microorganisms.
Preliminary data have shown that biofilm may be produced, and/or up-regulated, in *S. pyogenes* in response to either antibiotic treatment, other therapeutic molecules or environmental stimuli. In particular, subMIC antibiotic concentrations appear to stimulate biofilm formation; such phenomenon is being observed with increasing frequency for a number of microorganisms, both for biofilm and other virulence factors. The latest findings on what it is known on biofilm produced by *S. pyogenes,* its possible role in the pathogenesis of streptococcal infections, and on the interactions between antibiotics and other therapeutic molecules and streptococcal biofilm, will be examined.

S524 Diagnosis and treatment of *Aspergillus* biofilms

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Aspergillus fumigatus causes infections in both immunocompromised hosts and patients with chronic lung infections.
The initial establishment of *A. fumigatus* infections involves the germination of conidia and subsequent hyphal invasion of the lung tissues. Histology and microscopic examination of bronchopulmonary lavage samples reveals the presence of numerous *A. fumigatus* hyphae in the form of a complex mesh like structure, similar to other fungal biofilms.
We have developed an *in vitro* model of an Aspergilus fumigatus biofilm. This model possesses the classical elements of biofilm growth, namely multicellularity, matrix production and sessile resistance and shows has distinct developmental phases both genotypically and phenotypically.
I will discuss the *in vitro* antifungal activity of voriconazole, amphotericin B and caspofungin and how this model may relate to the use of antifungals in the clinic. Also how a better understanding of the biology of *Aspergillus fumigatus in vitro* may lead to a different approach to diagnosis of invasive fungal infection.

Challenges and solutions for determining pharmacokinetics of antimicrobials in the human body

S525 Imaging and microdosing – how do they fit for antimicrobial agents?

O. Langer (Vienna, AT)*

Most drugs exert their effects not within the plasma compartment, but in defined target tissues into which drugs have to distribute from the central compartment. Unfortunately, a complete and lasting equilibration between blood and tissue cannot always be taken for granted. Drug distribution processes may be characterized by a high intertissue- and intersubject variability and target site drug levels may substantially differ from corresponding plasma levels. Suboptimal target site concentrations may have important clinical implications, as it is a potential explanation for therapeutic failures. In particular for antimicrobial agents knowledge of drug tissue distribution is of primal importance as tissue drug exposure has been shown to be directly related to outcome of therapy. Therefore, determination of drug tissue penetration plays an important role in antimicrobial drug development. Positron emission tomography (PET) is nuclear imaging method, which can be used to study the tissue distribution of drugs labeled with positron-emitting radionuclides, such as carbon-11 or fluorine-18, non-invasively. These types of studies have been termed PET-microdosing studies, as the amount of drug administered in a PET study is usually less than 100 microgram. Due to low administered drug doses, the potential toxicological risk to human subjects is very limited. Consequently regulatory authorities require reduced preclinical safety testing as compared to conventional phase 1 studies. In the present talk, recent applications of PET-microdosing in antimicrobial drug development will be reviewed.

S527 PK modelling: obtaining PK profiles despite sparse sampling

J.B. Bulitta, A. Bingölbali, C. Landersdorfer (Albany, US)*

Objectives: Tissue penetration studies pose a significant challenge for pharmacokinetic/pharmacodynamic (PK/PD) modelling, especially if only one tissue sample can be obtained per patient. We sought to systematically evaluate the ability of various optimized and non-optimized clinical trial designs to determine the rate and extent of tissue penetration and their between subject variability (BSV).

Methods: Monte Carlo simulations with a 2-compartment model were performed in Berkeley Madonna to simulate plasma and tissue concentrations of 1000 patients for intravenous moxifloxacin as an example drug. Simulation scenario A assumed that the tissue concentration is proportional to the concentration in the peripheral compartment. For scenario B, rate and extent of tissue penetration were estimated and tissue volume fixed to a small value. Population optimal sampling times were determined in WinPOPT (V1.2). For 12 sampling schedules, 100 datasets of 48 patients were drawn randomly and analyzed by the MC-PEM algorithm in S-ADAPT V1.56 using the true model (2400 population PK analyses in total). Bias and precision of mean population PK parameters and variances representing BSV were calculated.
Results: Except for designs that sampled both plasma and tissue at the same time in all patients, in scenario A bias was <8% and precision <19% for population means and bias was generally <25% and precision <30% for BSV for all PK parameters. For scenario B and designs with 5 optimized plasma sampling times and one tissue sample per patient: (1) Designs with all tissue samples at the same time showed bias up to 48% for extent of tissue penetration and up to 78% for rate of tissue penetration. (2) A design with random tissue sampling times had bias <7% and precision <21% for all population means. (3) A design with four groups (each: n = 12) of patients each sampled at one optimized tissue sampling time achieved bias <3% and precision <13% for all population means and bias <18% and precision <33% for all BSV estimates.
Conclusions: Tissue samples should be obtained at multiple optimized time points to determine the rate and extent of penetration, even if only one tissue sample per patient can be obtained. Designs with serial plasma samples at population optimal times were beneficial. Designs with one plasma and one tissue sample per patient all at the same time performed poorly and benefitted from a full Bayesian approach for PK analysis.

S528 Role of pharmacokinetics in early stages of antimicrobial development

U. Theuretzbacher (Vienna, AT)*

The importance of diligent pharmacokinetic (PK) profiling in the early drug research and development stages to reduce late attrition rate has been increasingly recognized over the last decade and major advances have been made. Based on the results of ADME screening (absorption, distribution, metabolism, and excretion) during the lead optimization phase, relevant predictive information on the pharmacokinetic behavior of a preclinical candidate is typically obtained in two or three animal species before administration to humans. The preclinical PK assessment provides the input for *in vitro* and *in vivo* PK/PD (pharmacodynamic) models that evaluate exposure-effect relationships. Such PK/PD models are powerful tools for dose selection for the clinical phases of drug development. If phase 1 PK data are available, population based PK/PD models support effectively dose selection for late stage clinical trials. Moreover, a PK/PD guided approach can provide decision support for susceptibility breakpoints as well as strategies to mitigate resistance development. Recent late stage clinical failures illustrate the importance of understanding the impact of PKs such as protein binding and concentrations at infection sites and incorporating them early into adequate PK/PD evaluations.

Management of infections caused by viruses in haematopoietic stem cell recipients

S531 Epstein–Barr virus infections

J.M. Middeldorp (Amsterdam, NL)*

Epstein–Barr virus (EBV) infection in immune suppressed transplant recipients is a frequent and potential life-threatening complication. EBV causes B-cell lymphoproliferative disorders, collectively named PTLD, which may lead to malignant lymphoma if left untreated. The incidence, clinical appearance and severity of PTLD may be quite variable, requiring appropriate risk-stratification at onset and accurate and standardized diagnostic monitoring approaches in the post-transplant period aiming at early identification of EBV involvement and distinction from rejection and other (infectious) complications.
The biology and pathogenesis underlying PTLD in solid organ (SOT) and stem cell transplant (SCT) recipients has different features, with implications for diagnosis and treatment. Primary infection in the SOT and nearly all (re)active EBV infections in SCT pose the highest risk for fatal complications. A distinction should be made between early and late-onset PTLD, the former being directly EBV driven and frequently reversible by simple therapeutic intervention. The latter should be seen as a consequence of misdiagnosed early EBV-driven B-cell amplification, allowing additional genetic defects to accumulate, thereby increasing the malignant phenotype, which frequently require more severe intervention strategies, including chemotherapy.
Early PTLD can be recognized by dynamic increases of EBV-DNA load in blood over relatively short time intervals. In SOT this EBV load is usually cell associated, whereas in SCT both cell and plasma DNA loads are observed. Whole blood may be a preferred well standardized sample for diagnostic monitoring. Dependent on the type of transplant, therapeutic intervention may vary from a (mild-moderate) reduction of immunosuppressive treatment (IST), via use of Rituximab (rtX) to infusion of *ex vivo* activated EBV-reactive T-cells. A persistent decrease of EBV-DNA load may be taken as a sign of therapeutic efficacy, and monitoring EBV-reactive T-cells by tetramer FACS or ELISPOT tests may provide information on patient's immune capacity to conquer EBV-driven B-cell proliferation and restore EBV latency on the long term. Treatment failure is indicated by persistent increases of EBV-DNA over time, and should be taken as sign for more drastic intervention.
During early PTLD EBV-infected cells frequently disseminate via the circulation, and thus becomes easily detectable, whereas late PTLD may be confined to tissue with little circulating cells. EBV transcription profiling has revealed differences EBV gene expression in biopsy material and circulating EBV-carrying cells in patients with early PTLD, suggesting transcription regulation in affected lymphoid tissues. Furthermore analysis of EBV gene expression in PTLD lesions at the single cell level, reveals heterogeneous EBV gene expression, reflecting more complex underlying pathogenic events rather than simple proliferation of latency-III (growth program) expressing B-cells.
Infrequent measuring of EBV DNA loads is not considered a proper diagnostic approach, because mere EBV DNA levels may vary between patients. These generally have no clinical implication when being stable over time. It is suggested that frequent (weekly) measurement of changes in EBV-DNA load at early times post-transplant using sensitive and standardized techniques, coupled to appropriate and timely EBV load-guided therapeutic intervention may reduce and even prevent PTLD in the transplant setting.

S532 Adenovirus infections

A. Heim (Hanover, DE)*

Human Adenoviruses (HAdV) are a highly genetically divergent group of DNA viruses consisting of seven species with 54 types. A few of these HAdV types (1, 2, 5 and 31) are clearly associated with infections of immunosuppressed patients as for example haematopoietic stem cell recipients. These HAdV types are not typical opportunistic agents but also cause less severe diseases in immunocompetent patients.
HAdV can persist for several months to years after an acute infection even in immunocompetent hosts and are more prevalent in children than in adults. Complications due to HAdV in haematopoetic stem cell recipients may originate from HAdV persistence and de novo infections. Due to HAdV persistence, diagnosis of HAdV disease cannot be made by mere detection of HAdV DNA. In case of organ related HAdV diseases, diagnosis is feasible by HAdV detection at the putative infection site, e.g. in case of cystitis by HAdV detection in urine. HAdV infection can also lead to potentially fatal disseminated

HAdV disease. Main risk factors are young age, lymphopenia, T cell depletion and high dosage of immunosuppressive drugs. Diagnosis of the disseminated HAdV disease has been simplified by HAdV load testing in peripheral blood samples using quantitative HAdV PCR. High virus loads (>1e6 genome equivalents/ml) were clearly associated with disseminated disease whereas low virus loads (<1e4 genome equivalents/ml) can be observed in case of HAdV latency. However, a precise threshold HAdV load value has not yet been determined. Disseminated disease may be predicted in case of rapidly rising HAdV loads in blood.
Pathophysiology of HAdV disseminated disease includes massive virus replication in affected organs as for example the liver which may be infected by interaction of HAdV capsids with clotting factor X. Furthermore binding of HAdV capsids to platelets, formation of immune complexes with preformed HAdV specific antibodies as well as induction of cytokines may contribute to the pathophysiology of disseminated HAdV disease.
An antiviral treatment for HAdV disease has not yet been established. Cidofovir may have beneficial effects if applicated early but may fail if applicated in case of very high virus loads. HAdV specific T-lymphocytes seem to be a promising therapeutic approach because risk of disseminated HAdV disease is clearly associated with lymphopenia. However, HAdV specific T lymphocytes must be prepared before onset of disease in order to be available in time.

What do we expect from MALDI-TOF?

O533 Elaborate MALDI-TOF MS-based identification of micro-organisms: the Saramis concept

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Identification of microbial isolates by MALDI-TOF MS of whole cells gains more and more acceptance in clinical microbiology. The reliability of this technology depends largely on the reference database and the algorithm for spectral comparison. Most simply, a sample's spectrum is directly compared to spectra of reference strains and as a result a rated list of matches is provided. A more elaborate approach is realized in the (Spectral Archive and Microbial Identification System (SARAMIS). SARAMIS uses a comprehensive database of mass spectral data of reference strains for automated identification. Two types of spectral data are contained in the database: so called SuperSpectra for fully automated first-line identification (including result transmission) and Reference Spectra for semi-automated second-line identification. The concept of SuperSpectra has been developed in consideration of the natural, intra-specific diversity encountered in all microbial species. This diversity is also reflected in mass spectral fingerprints of individual isolates as variations in peak patterns, which complicates the selection of appropriate reference data. To compute SuperSpectra, spectral fingerprints of a number of 10–20 isolates of a species are searched for conserved mass signals that are summarized in a consensus spectrum. In a second step, each mass in the consensus spectrum is compared to the entire reference database to establish its specificity at different taxonomic levels. By doing so, mass signals can be selected that are specific at a desired taxonomic level, e.g., species-specific. By excluding masses from the consensus spectra that are specific only at higher taxonomic levels, the pattern of the remaining masses is highly specific. This makes random matches highly improbable, practically excluding false identifications. The SARAMIS database contains at present 2700 SuperSpectra, representing 900 microbial species. Since SuperSpectra by definition represent typical isolates of a species (not to be confused with type strains), a certain percentage of isolates of a given species will not be captured in automated routine analysis. In this case, the second-line identification starts that applies a direct comparison to all 35,000+ Reference Spectra in the database (representing 1500 species). By this two-step approach basically all clinically, most veterinary and, increasingly, environmental isolates can be reliably identified by a largely automated procedure.

O534 MALDI-TOF MS for identification of routine bacterial isolates in a clinical microbiological laboratory

T. Jensen, B. Gahrn-Hansen (Odense, DK)*

Objectives: To compare identification of routine bacterial isolates using the SARAMIS identification system and conventional methods.
Methods: Consecutive bacterial isolates from urine- and bloodcultures were identified using conventional biochemical methods or VITEK II. All isolates were subsequently identified using MALDI-TOF (AXIMA@SARAMIS®). Discrepant isolates will be further identified by means of 16S RNA sequencing.
Results: A total of 3,695 isolates (1,095 from blood, 2,600 from urine) were included. 71.8% of the isolates showed fully agreement on species-level, for another 9.7% fully agreement on genus-level was achieved. 15.7% of the isolates could not be identified by MALDI-TOF using the 95% identification-score.
In 2.8% of the isolates, the two methods gave different identification. These isolates are currently subject to further characterization by 16S RNA sequencing.
Conclusion: MALDI-TOF (AXIMA@SARAMIS®) gives a fast and reliable identification of commonly encountered bacterial isolates in a clinical microbiology laboratory but has certain limitations. Enterobacteriaceae (apart from *Shigella*), *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Haemophilus* influenza, *Staphylococcus*, *Enterococcus faecalis* and *E. faecium* could usually be identfied to species level. Anaerobes and streptococci were often unidentified.

O535 Fundamental improved sample preparation technique for direct and fast analysis of positive blood cultures

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Objectives: Automated blood cultivation of patients with suspicion for sepsis is a routine clinical approach. Fast and accurate species identification of microorganisms after signalling of growth is of fundamental interest. Currently, the identification takes up to two days because of the necessity for sub-cultivation and biochemical identification. The presented simple protocol has the potential to shorten the identification time to minutes.
Methods: Blood cultures spiked with bacteria or yeast were used for method establishment, inoculated blood cultures and routine samples to optimize and validate the protocol. 1ml of a blood culture was mixed with 200µl of lysis solution followed by vortexing and centrifugation. Thereby, blood cells were disrupted but bacteria and yeast survived. Supernatant was removed and the pellet carefully suspended in wash solution. After centrifugation supernatant was removed and bacteria from the pellet were transferred to a MALDI target directly or after a short extraction procedure. Species ID was achieved by measurement in a MALDI-TOF MS mass spectrometer followed by analysis with the MALDI Biotyper software. The method was optimized by varying volumes and ratios of blood culture liquid and lysis solution.
Results: Positive blood cultures showed an average cell density of about 10^7 cfu/ml and a high number of blood cells. The presented protocol was able to remove blood cells extensively without significant loss of microorganisms. Therefore, resulting MALDI-TOF spectra were of high quality. Different bacterial species (enterobacteria, Gram negative non-fermenting bacteria, staphylococci, enterococci, streptococci, *Haemophilus* sp.) could be identified with the procedure, reliably. Also yeasts which could not be detected with previously published protocols were identified. In some cases an identification was possible even 1–2 h before blood cultures were flagged positive by the automate, showing the high sensitivity of the method. Mixed cultures in most cases lead to non-identification or observation of only one species. Work on bioinformatic algorithms will improve this.
Conclusion: The new protocol for species identification from positive blood cultures could be demonstrated as a very fast and accurate alternative to classical methods. Identification time is shortened from

days to minutes for the majority of samples, thereby enabling quicker adoption of antibiotic therapy.

O536 MALDI-TOF MS for direct bacterial identification from positive blood culture pellets

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Objectives: Blood cultures are the best approach to establish the etiology of bloodstream infections. Rapid identification of etiological agent of such severe infections is pivotal to guide antimicrobial therapy. The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) allows the identification at the species level in few minutes of bacteria by measuring molecular masses of proteins from whole bacterial extracts. We applied a simple procedure for lysing erythrocytes from positive blood cultures and prepared a bacterial pellet for MALDI-TOF MS analysis.
Methods: Pellets from positive blood culture vials detected by Bactec 9240 (Becton Dickinson) were prepared by using an ammonium chloride lysing solution and centrifugation steps. Pellets were then analyzed by MALDI-TOF either by direct deposition on the target plate or after a formic acid:acetonitrile extraction step.
Mass spectra were acquired using the Microflex MALDI-TOF MS (Bruker Daltonics). The identification was considered as valid at the species level when the score value was >2, as valid at the genus level when the score value was >1.7 and <2 and as not valid when the score was <1.7. The identifications obtained with MALDI-TOF MS analysis were compared with biochemical identification.
Results: 122 monobacterial positive blood vials from 76 patients were analyzed. 96 (78.7%) bacterial identifications were obtained by MALDI-TOF MS analysis, of which 69 (56.6% of 122) exhibited a score value >2 and 27 (22.1%) a score value >1.7 and <2. Regardless of the score value, 95 (98.95%) of the 96 bacterial identification obtained with MALDI-TOF MS were correct at the species level and 1 correct at the genus level only. In 26 (21.3%) of cases, no reliable identification was obtained (score value <1.7). 21/26 bacteria (80.8%) were Gram positive, mainly streptococci (n = 13) and coagulase-negative staphylococci (n = 5). Most unidentified streptococci were *Streptococcus pneumoniae*. Among the 5 Gram negative bacteria with a score <1.7, 4 were of encapsulated species (2 *Klebsiella pneumoniae* and 2 *Haemophilus influenzae*).
Conclusion: The use of an ammonium chloride-driven hemolysis before analyzing directly positive blood cultures by MALDI-TOF MS is a very promising new method allowing fast, accurate and inexpensive identification of the etiological agents of life-threatening bloodstream infections.

O537 Direct identification and rapid antimicrobial susceptibility testing of bacteria from positive blood culture bottles by using MALDI-TOF MS and Phoenix rapid AST cards

T. Adam, C. Gröger, U. Göbel (Berlin, DE)*

Objectives: Early and appropriate antimicrobial therapy critically determines the outcome of bloodstream infections. Rapid identification (ID) and antimicrobial susceptibility testing (AST) is, hence, mandatory to adjust empirical antimicrobial therapy instituted during the first two hours after onset of symptoms. To reduce the turnaround time (TAT) aliquots from positive blood culture bottles (BC) were subjected to differential centrifugation and gel separation using BD Vacutainer SST II tubes. The preparations were used for direct identification by MALDI-TOF mass spectrometry (MS). In addition, we are optimizing a protocol for rapid AST using the Phoenix BD system.
Methods: Blood cultures were drawn from septic patients and incubated using the Bactec BD blood culture device. In the rapid arm aliquots from consecutive positive BC bottles were processed for direct identification by Mass Spectrometry using the MALDI Biotyper (Bruker) system. Controls were processed according to standard procedures, inoculation of solid media and subsequent ID and AST using the MALDI Biotyper or the Vitek 2 system (bioMérieux), respectively. In case of discrepant ID results isolates were subjected to 16S rRNA gene sequencing..
Results: So far, we have tested 115 positive BCs. Direct MALDI TOF analysis resulted in 76 (66%) species identifications showing no discrepant results when compared with identification of colonies grown from these BCs. In 9 BCs more than 1 isolate could be grown on plates. Thus, in 73% of monoinfectious BCs MALDI TOF can reveal the etiologic agent in less than 1 h.
Conclusion: As compared to conventional procedures the combined use of MALDI-TOF MS and rapid AST may significantly reduce the TAT in diagnosis of blood stream infections..

O538 Speeding up identification of bacteria from urine by the use of an Alifax HB&L Uroquattro incubator followed by Maldi Biotyper identification

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Objectives: Direct identification of bacteria from infected urine by the MALDI-Biotyper (MBT) has been shown previously. To enable quantification as well as identification, which is necessary for routine diagnostic we have tested the combination of an automated quantification system, the Uroquattro (ALIFAX HB&L), with the MALDI-TOF workflow.
Methods: For this proof of concept study, 161 urine samples from routine were analysed in parallel.
For the novel combined workflow, a 500 µl aliquot was transferred into a 2ml HB&L tube with nutrient broth and stirrer, placed into the Uroquattro instrument for 3 h at 37ºC and the CFU value was read. Turbidity of the vials is monitored and bacterial count is calculated. After incubation, 500 µl aliquots from the positive vials were pipetted onto a 100 µl Ficoll-Paque Plus cushion and centrifuged (5 min at 15000 g in a 1.5 ml Eppendorf cup). Supernatant was discarded and part of the pellet transferred onto a MALDI target, air dried, overlayed with matrix solution (HCCA), again air dried and subjected to identification by mass spectrometry using the MBT system. This approach was compared to our standard workflow: plating the urine on 2 Petri dishes in a quantitative way, MBT identification of colonies after over night incubation at 37ºC.
Results: A bacterial count above 10E4 CFU was obtained for 77 of the 161 samples. These patients where considered to have a bacterial infection. The bacterial count was identical to a large extend with both methods. However, MBT identification was only possible in 63 of the samples incubated in the HB&L. Here, the results matched those of the standard protocol. This was due to the presence of more than one pathogen (as shown by the standard workup) which the current identification software of the MBT can not resolve.
Conclusion: This protocol enables reliable bacterial identification together with a bacterial count in few more than 3 hours in cases of mono-bacterial infection using the standard protocol of the HB&L. Ongoing improvement to the MBT identification algorithms will resolve the problem of poly-bacterial infections. Further extension of this approach to usually sterile body fluids, tracheal secretions and pleural aspirates also here may lead to a dramatic reduction in turnaround time of microbiological labs for identification and subsequent AST-testing.

O539 Standardized method for fungal identification using liquid culturing and MALDI-TOF MS profiling

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Objectives: MALDI-TOF fingerprint analysis became a valuable tool for microorganism identification and classification, recently. While for bacteria and yeasts simple and robust approaches for sample preparation have been reported, leading to reproducible results with low impact of cultivation conditions and growth state, the situation for filamentous fungi keeps more complicated. In particular, sample preparation of molds with rigid cell walls is more challenging and the fingerprint spectra derived from spores and mycel show significant differences. Also fingerprint spectra seem to be dependent on cultivation media.

We present a novel approach for sample preparation of fungi prior to MALDI-TOF MS improving reproducibility and quality of spectra, significantly.
Methods: Fungal species (e.g. *Aspergillus* spp., *Fusarium* spp.) harvested from solid media were grown over night in liquid medium. Subsequently, cell material was harvested by centrifugation, and washed with water. The pellet was dried well and extracted using 35% formic acid/50% acetonitrile. 1µl of the extract was spotted onto a MALDI target, dried on air, and overlaid with HCCA matrix. Mass spectra were acquired in the linear mode, mass range 2000 to 20000 Da, using a microflex MALDI-TOF mass spectrometer (Bruker Daltonik GmbH, Bremen). Spectra were analysed using the flexAnalysis software, reference libraries were created and bioinformatic analyses were performed with the MALDI Biotyper 2.0 software (Bruker Daltonik).
Results: Culture in liquid medium has lead to mycelia without spores and thereby largely homogenous cell material. These samples facilitated a successful protein extraction by a simple, short standardised method. Mass spectra generated based on the novel sample preparation method were reproducible and contained many characteristic peaks, thereby leading to a significantly improved identification security. Further, the higher quality of spectra increased the differentiation power of the method and could highlight subspecies differences. A core database of 50 filamentous species has been established and used for first comparative studies. Technical and biological replicates could be identified successfully as well as isolates not included in the database. Reproducibility of results obtained with different mass spectrometers could be demonstrated.
Conclusions: The presented approach may lead to significant improvement of fungal identification in clinical or other routine applications.

O540 Diagnosing fungal infections by mass spectrometry

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Objectives and Method: Mass spectrometry is presented as a modern analytical tool for fungal strain typing and diagnosing fungal infections.
Results: Qualitative and quantitative proteomics approaches are documented on various *Aspergillus* strains: virulence protein factors present on fungal spores are identified by peptide mapping, peptide- and de novo-sequencing. Quantitative proteomics is addressed by NOVA-Q in house-developed software improving the precision of results in samples labeled by SILAC. Metabonomics approach is illustrated by the detection of minor macrolide antibiotics produced by Streptomyces strains. Peptidomics is represented by tracking non-ribosomal cyclic peptides and depsipeptides produced by Beauveria, Paecilomyces and Pseudallescheria genera. Peptide profiles are used as chemotaxonomic tools. Patented unique non-ribosomal lasso-peptide structures are reported as extremely specific fungal markers. Glycomics and lipidomics armory is illustrated by hexosylceramide analysis in *Scedosporium* by Fourier-transform ion cyclotron resonance mass spectrometry. Clinical samples (tissues, whole blood) handling by advanced ambient ionization techniques is reported with special focus to lipids in brain, eye bulb and lungs (murine, porcine). The fungal infections in plants is addressed by DAPPI mass imaging.
Conclusion: The current advances in mass spectrometry will lay the experimental foundation for modern sensitive diagnostic tools. We predict that particularly mass imaging of tissues infected by molds will lead to discovery of specific fungal biomarkers.

O541 Comparative evaluation of MALDI Biotyper system, manual biochemical tests and BD Phoenix™ automated microbiology system for species identification of staphylococci

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Objectives: *S. aureus* and several species of coagulase-negative staphylococci are common human pathogens. Therefore, comprehensive and accurate identification of *Staphylococcus* species is of great importance. Several ID methods based on conventional physiological or biochemical characterization are commercially available in manual and automated formats. The presented study aimed to examine the accuracies of manual identification tests, BD Phoenix™ system and MALDI Biotyper system for the discrimination of *Staphylococcus* species.
Methods: A total of 301 clinical isolates of staphylococci were studied. For conventional species identification, manual or semi automated biochemical identification systems (bioMérieux, France) were used. All isolates were also identified by Phoenix™ automated microbiology system (BD Diagnostic Systems, USA) as well as by MALDI Biotyper technique (Bruker Daltonik GmbH, Germany) which is based on fingerprinting of mass spectra acquired from fresh bacterial cultures. 16S rRNA gene sequencing was performed as confirmatory approach for discordant cases.
Results: Clinical isolates from 31 regions of the Russian Federation were investigated including 101 isolates presumably identified by conventional microbiological methods as coagulase-negative staphylococci and 200 isolates reported as *S. aureus*. A discordance between conventional methods of identification and the other tested technologies was found in 9 (2.9%) cases for MALDI Biotyper technique, and in 29 (9.6%) ones for BD Phoenix™ automated system. Discordant results of ID using the Biotyper and Phoenix were obtained for 24 (8.0%) isolates. Unambiguous species ID was achieved by MALDI Biotyper technique and Phoenix™ automated system in 292 (97.0%) and 277 (92.0%) cases, respectively. Likewise, 8 (3.8%) and 21 (7.0%) isolates, respectively, were identified at the genus level only. Two isolates (0.7%) did not belong to the *Staphylococcus* genus according to both approaches. The discrepant cases were analyzed by 16S rRNA gene sequencing which confirmed the benefits of MALDI Biotyper ID results in most of the cases.
Conclusion: The MALDI Biotyper technique was superior to the manual and automated (BD Phoenix) biochemical tests for the species identification of staphylococci.

O542 Comparison of MALDI-TOF MS, the Vitek 2 Anaerobic card and BD BBL Crystal Anaerobe ID kit for identification of anaerobic bacteria

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Objective: Compare MALDI-TOF mass spectrometry (MS) with established automated identification systems for anaerobic bacteria. All methods were compared to 16s rDNA sequencing.
Methods: 66 isolates from our routine diagnostic laboratory cultured on non-selective media to secure pure culture, aged no more than 24–48 hours, underwent Gram staining, catalase testing and spot indole if requested by the identification method. Otherwise standard procedures as specified by manufacturer were followed for VITEK 2 Anaerobic and Corynebacteria identification with VITEK 2 and the BD BBL Crystal Anaerobe ID kit with the BBL autoreader. For sequencing of the 16s rRNA gene, DNA was extracted using the Prepman Ultra protocol (Applied Biosciences) and thereafter performed according to standard procedure using an ABI 3730 sequencer and the BLASTn search tool. Identification of the microorganisms by MS was performed using the Microflex MALDI-TOF mass spectrometer (Bruker Daltonik GmbH) with the Biotyper 2.0 software. Preparation of the bacteria was performed using the ethanol/formic acid extraction procedure according to the manufacturer (Bruker Daltonik GmbH). All bacteria were analyzed in

duplicates. The Biotyper 2.0 software generates a list of species matches ranked by a log score value. In this study we accepted the score value of 1.7 and higher if the duplicates matched each other.
Results:36 different anaerobic species were identified. Proportion of isolates identified correctly according to species group compared to 16s rDNA sequencing are presented in table 1. Generally MALDI-TOF MS performed better than the 2 automated systems, although short comings were present for some species. Of the two automated systems the VITEK2 Anaerobic and Corynebacteria identification card performed marginally better than the BD BBL Crystal™ Anaerobe ID kit.
Conclusion: The automated systems for anaerobic identification show limited performances. Although the MALDI-TOF MS performed better, significant shortcomings are also present. The latter could be explained by database limitations of the MALDI Biotyper 2.0 software, i.e. several subspecies of *Fusobacterium nucleatum* have been described but are not included. At present time 16s rDNA sequencing remains the gold standard but may be somewhat replaced by MALDI TOF MS in the near future depending on further development of the database. The 2 automated identification systems can be used in adjunction to more conventional identification.

Table 1. Proportion of anaerobic isolates correctly identified by MALDI-TOF mass spectrometry, BD BBL Crystal™ Anaerobe ID kit and the VITEK® 2 Anaerobic and Corynebacteria identification card according to species groups

	16s rDNA sequencing	MALDI-TOF MS	Crystal	VITEK 2 ANA
Actinomyces spp.	5/5	3/5	0/5	2/5
Bacterioides spp.	8/8	7/8	2/8	2/8
Clostridium spp.	16/16	13/16	9/16	10/16
Corynebacterium spp.	7/7	3/6	2/6	2/6
Fusobacterium spp.	10/10	6/10	3/10	4/10
Prevotella spp.	5/5	4/5	2/5	3/5
Propionibacterium spp.	10/10	10/10	6/10	10/10
Other	5/5	2/5	3/5	4/5

Current emerging bacterial and viral infections

O543 Eco-epidemiology and complete genome comparison of bat SARS coronavirus in China reveal bats as reservoir for frequent recombination

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Objective: Despite the identification of SARS-CoV-like viruses in horseshoe bats in China, the evolutionary and possible recombination origin of SARS-CoV remains undetermined. To better define the epidemiology and evolution of bat-SARS-CoV in China and their role as recombination origin of SARS-CoV in civet, a four-year study on coronaviruses in Chinese horseshoe bats in Hong Kong and Guangdong province of southern China was conducted.
Methods: Respiratory and alimentary samples were collected from 1401 Chinese horseshoe bats captured in Hong Kong and Guangdong province of southern China over a four-year period and were detected for coronaviruses by reverse-transcriptase polymerase chain reaction. Five hundred and eleven bats from Hong Kong were also tagged to study the migration pattern of bats and viral persistence. The complete genomes of 10 strains of bat-SARS-CoV obtained at different time were sequenced and compared to the previously characterized genomes.
Results: Bat-SARS-CoV was detected in alimentary specimens from 130 (9.3%) bats, with peak activity during spring. Bats carrying the viruses appeared healthy, with viral clearance occurring between two weeks to four months. Tagging exercise showed that migration distances of Chinese horseshoe bats in Hong Kong range from 1.86 to 17 km. Complete genome sequencing of 10 strains of bat-SARS-CoV revealed frequent recombination between different strains, especially among the bat viruses in China. Recombination was detected between bat-SARS-CoV Rp3 from Guangxi and Rf1 from Hubei in the possible generation of civet SARS-CoV SZ3, with breakpoint at nsp16/spike region.
Conclusion: Bat-SARS-CoV causes acute, self-limiting infection in horseshoe bats which serve as reservoir for recombination between virus strains from different geographical locations within reachable foraging range. Civet SARS-CoV is likely a recombinant virus arising from bat-SARS-CoV strains closely related to Rp3 and Rf1. Such frequent recombination in these animals may have accounted for the cross-species transmission and emergence of SARS.

O544 Epidemiology and control of Q fever in the Netherlands, 2007–2009

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Objectives: Q fever is caused by *Coxiella burnetii*. Sheep and goats are frequently described as the source. Humans are usually infected through inhalation and half of the infected show symptoms. Before 2007, Q fever was a rare disease in the Netherlands. Since 2005, Q fever emerged in small ruminants and subsequently in 2007 in the human population leading to the largest Q fever outbreak recorded up to date.
Methods: Analysis of data from public health facilities, regional laboratories, the Animal Health Service and the National Institute for Public Health and the Environment to describe the Q fever outbreak occurring in the Netherlands.
Results: In the spring of 2007, Q fever occurred in a village in the province of Noord Brabant with 168 cases. In 2008 and 2009 increasingly larger outbreaks occurred in an increasingly wider area. By November 2009, more than 3000 cases have been notified. Six patients have died. The number of chronic cases is not known. So far, contact with hay/manure and a house location close to an infected dairy goat farm have been identified as important risk factors for human Q fever. In the affected area dairy goat farming is common and goat density per square kilometre is the highest in the Netherlands. Since 2005 and preceding the human outbreaks, abortion waves caused by *C. burnetii* have been noticed on 27 dairy goat farms and 2 dairy sheep farms in the regions where most of the human cases occurred. Sampling of incriminated farms, animals and surroundings resulted in detection of *C. burnetii.* Preliminary MLVA analysis points at spreading of a single clone in goats, however the presence of this clone is not yet widely confirmed in humans. Control measures including mandatory veterinary notification as well as hygiene measures during spread of manure, lambing season and mass vaccination of goats and sheep are being implemented. Preliminary results of the monitoring of vaccination indicate a decrease of the abortion rate in the vaccinated groups to zero and a drop in bulkmilk levels of *C. burnetii* DNA.
Conclusion: The Q fever outbreak starting in 2007 in the Netherlands is still on going. It is mainly restricted to the south of the country in an area with intense dairy goat farming. However the epidemic has been expanding to other areas in 2009. The effect of control measures is still to be awaited. Epidemiological data point towards the intensive dairy goat farming as the main source of the outbreak.

O545 Evaluation of a diagnostic algorithm for acute Q-fever in an outbreak setting

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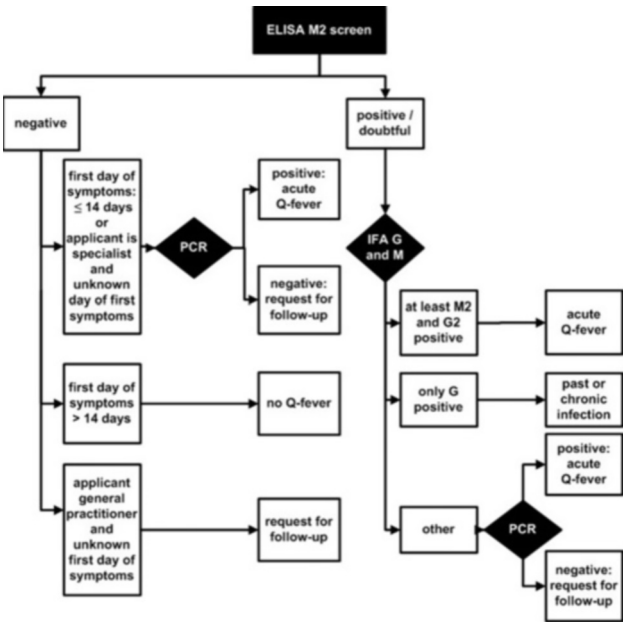
Objectives: An outbreak of Q-fever with over 3300 notified cases is ongoing in the Netherlands. Since 2007, immunofluorescence assay (IFA) has been the cornerstone of Q-fever serology in our hospital. IFA, however, is time-consuming and subject to inter-observer variability. Furthermore, the lag phase in antibody response to *Coxiella burnetii*

renders serology less suitable for diagnosing early disease. Alternative diagnostic approaches include an ELISA for IgM phase II antibodies as screening assay (M II screen). In addition, IS1111 PCR on serum samples is capable of diagnosing acute Q-fever before antibodies appear. In 2009, we introduced a diagnostic algorithm (figure) for acute Q-fever with the M II screen as initial step. Subsequently, IFA and/or PCR were performed depending on outcome of M II screen, date of onset of disease and inpatient or outpatient setting. When diagnostics were inconclusive a 14-day follow-up serum sample was requested. Here, we evaluated the value of the algorithm in an outbreak setting.

Methods: We retrospectively evaluated outcome of Q-fever diagnostics according to the new algorithm in all patients referred between May 15th and 31st, 2009, with date of onset of disease unknown or <3 months.

Results: In the 17-day period, 825 patients were tested. The diagnosis acute Q-fever was made in 256 patients (31%) – in 197 patients on the first serum sample, in 59 patients on the 14-day follow-up serum sample. A negative M II screen was obtained in 669/825 first serum samples resulting in reduction of IFAs performed by more than 80%. Ninety-two M II screen negative patients were diagnosed with acute Q-fever by positive PCR. Cross-reactivity was documented in 4% of patients with a positive M II screen. Almost 50% of physicians did not list date of onset of disease. Requested follow-up serum samples were not received from 306 patients leading to inconclusive outcomes.

Conclusion: Introduction of the M II screen significantly reduced the number of IFAs performed, while introduction of PCR allowed for diagnosis of acute Q-fever in a substantial number of seronegative patients. Pitfalls to the presented algorithm are the poor communication by many physicians of the first day of disease, which is a critical component in the algorithm, and the suboptimal response to requests for follow-up serum samples implying that cases of acute Q-fever might have been missed.



O546 Mortality burden of *Clostridium difficile*-associated disease in adults: an under-recognized crisis

T. Eckmanns (Berlin, DE)*

Objectives: In response to the increase of *Clostridium difficile* associated disease (CDAD) and the emergence of *Clostridium difficile* ribotype 027 in Germany in 2007, the Robert Koch Institute (RKI) implemented nationwide notification of severe cases of CDAD, starting from November 2007.

Methods: We used first years surveillance data to assess the population burden of CDAD by calculating years of life lost (YLL) for infections with *Clostridium difficile*. In addition, data from the national surveillance system were used to assess YLL for the most common gastrointestinal pathogens which are notifiable in Germany, i.e., *Campylobacter* spp., *Salmonella* spp., rotavirus, and norovirus. All fatal cases associated with these pathogens from 2004–2007 were included. For CDAD, we included fatal cases from 01/01/2008–31/12/2008. WHO life expectancy table (West Label 26) without discount and age-weighting was used to calculate sex-weighted YLL for 19 age groups with a cut-off at age 85+ years.

Results: A total of 419 cases of severe CDAD, including 224 fatalities, were reported to the RKI. Age dependent YLL are shown in figure 1. Overall, CDAD constitutes 66% of all YLL among the included gastrointestinal diseases.

Conclusions: Substantial underreporting of severe and fatal CDAD should be expected, since reporting is physician-based so far. With steeply increasing case numbers in Germany, our result is alarming. YLL from CDAD is an important measure of population burden and should be considers when allocating public health resources. To determine trends in occurrence of more toxigenic strains and their impact in the community and in the hospital setting, continued surveillance for CDAD is compulsory on an international scale.

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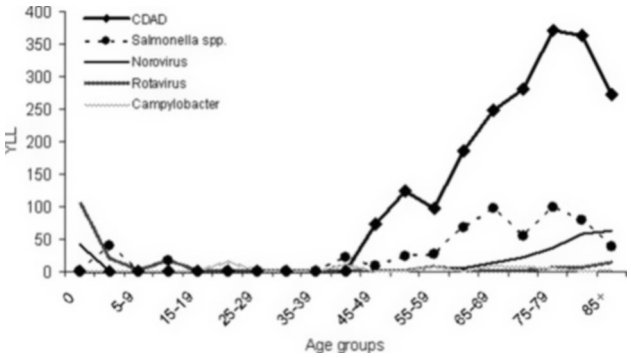

Figure 1. YLL for gastrointestinal infections in Germany.

O547 Proposition of an algorithm for the toxigenic *C. difficile* diagnosis

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Objectives: To evaluate a new test that detect *C. difficile* –enzyme glutamate dehydrogenase antigen (GDH) and toxin A/B in one step using the culture, like reference method; and to propose an algorithm for the routine diagnosis of CDAD.

Methods: CDAD study was performed to 970 stool samples of patients more than 1 year old, between February and October 2009. It was studied:

– Detection of GDH and Toxin A/B by ICT Techlab® C.diff Quick chek Complete (Inverness Medical)
– Culture on CLO plates (Biomerieux).

When GDH(+), toxin(−) and culture(+): direct toxin detection from culture was performed. When GDH(+), toxin(−) and culture(−): spore selection by alcohol treatment was performed and cultured on CLO plates.

Results: The 90.2% of samples were (−) to GDH, toxin and culture (875); and the 3.4% (33) (+) to those parameters. The 4.8% (47)

were GDH(+), toxin(−) and culture(+), being the 48.9% (23/47) of those samples toxin(+) when direct toxin detection from culture was performed. The 1.3% (13) were GDH(+), toxin(−) and culture(−), recovering by alcohol treatment the 46.1% (6/13) of those samples, being the 15.4% (2/13) toxin(+). The 0.1% (1) was GDH(+), toxin(+) and culture(−); and the 0.1% (1) GDH(−), toxin(−) and culture(+).
The prevalence of toxigenic CD was 6.1%. The S, E, VPp and VPn of GDH compared to culture were: 98.7%, 97.9%, 81.6% and 99.8%.
The algorithm purpose is:
GDH(−)/toxin(−): not CD
GDH(+)/toxin(+): toxigenic CD
GDH(+)/toxin(−): culture. If culture(+): direct toxin from culture. If toxin(+): toxigenic CD; if (−): non toxigenic CD. If culture(−): alcohol treatment. If culture(+): toxin. If toxin(+): toxigenic CD; if (−) non toxigenic CD. If culture(−): not CD.
Conclusions: This test is reliable, sensitive and specific for detect CD from stool. The detection of direct toxin from culture allowed to recover the 48.9% of GDH(+)/toxin(−) samples, increasing the prevalence of toxigenic-CD from 3.4% to 5.8% and with alcohol treatment to 6.1%. This algorithm allows to exclude CD without additional tests when GDH is (−) (90.2% of samples). In 93.6% of samples the results can be obtain in less than 2 hours.

O548 Risk factors and outcome of *Clostridium difficile* infection due to the four predominant PCR-ribotypes in the Netherlands

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Background: *Clostridium difficile* infection (CDI) due to hypervirulent PCR-ribotypes (types) have been well described. Little is known about other frequently encountered types.
Methods: We studied risk factors and clinical characteristics of the four predominant types in The Netherlands, as observed by The National Reference laboratory at the Leiden University Medical Center, from May 2005 until October 2008.
Results: Among 2047 isolates, the four predominant types were 001 (n = 162; 8% of all isolates), 014 (n = 217; 11%), 027 (n = 305; 15%) and 078 (n = 205; 10%). Patients with types 014 and 078 were younger (age <65 years 39% and 35%, respectively) than patients with types 001 and 027 (26% and 22%, respectively). Type 027 patients less frequently had community associated (CA) CDI than patients with any of the 3 other types (8% vs. 19–25%). Use of clindamycin was a specific risk factor for type 001, when compared the other types (31% vs. 6–7%). Type 078 patients with complicated CDI were younger (<60 years) than patients with types 001, 014 and 027 (19%, vs. 0%, 9% and 11%, respectively). Attributable mortality for types 001, 014, 027 and 078 was 0%, 0%, 3% and 2%, respectively. Patients with type 027 most often had a recurrence >8 weeks after the first infection (10% vs. 3–4% for the 3 other types).
Conclusions: Risk factors for and the clinical course of CDI vary and depend on the involved type. The antibiotics associated with the highest risk of acquisition of CDI relate to the specific type.

O549 Crimean-Congo haemorrhagic fever, ticks and base measures

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Crimean-Congo haemorrhagic fever (CCHF) is a viral haemorrhagic fever of the Nairovirus, in the Bunyaviridae family of viruses. CCHF virus possesses a negative-sense RNA genome consisting of three RNA segments: the large (L), medium (M), and small (S) segments. For an arthropod-borne virus, the genomic plasticity of CCHF virus is surprisingly high. It seems likely that genetic reassortment may primarily occur during coinfection of ticks due to the transient nature of vertebrate infections relative to the long-term persistent virus infections seen in ticks and their obligate need to obtain blood meals at metamorphic junctures. Substantially, movement of genetic lineages of CCHF virus, particularly over greater distances and between regions not linked by livestock trade, likely also involves migratory animals or birds that are either infected or are carrying virus-infected ticks. Consequently, however migratory birds those mediate genetic lineages of CCHF exist critical point for CCHF struggle such as avian influenza, ticks should be targeted at first. Especially, fields with high risk should be out of order for pasturing and disinfected with repellent medicines. Acaricide treatment of livestock in CCHFV endemic areas is effective in reducing the population of infected ticks.

O550 Risk factors and pathological correlations in severe courses of hantavirus infections in Germany

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Objectives: Hantavirus infections show a high variability in the intensity of symptoms depending on the hantavirus strain, but also individual differences were observed. Gender- and patient-specific differences in susceptibility and severity of infectious diseases, especially for hantaviruses, have often been discussed. Therefore, we analyzed epidemiologic and clinical data to identify risk factors for severity.
Methods: Hantavirus-infected patients (18 men and 4 women) hospitalized in the Department of Nephrology from 2002 through 2008 were included. The diagnosis was confirmed by detection of circulating anti-hantavirus IgG- and IgM-antibodies. Demographic data, underlying diseases, biopsy, cardiac, ultrasound and laboratory findings were recorded.
Results: The men-to-women ratio for diagnosed hantavirus infections in Germany is 2.6:1. The higher incidence of infection in men seems to be caused by the way of transmission and corresponds with other rodent-borne zoonoses. Gender or pre-existing conditions did not influence the clinical presentation. Thrombocytopenia precedes organ failure and severe courses were associated with lower platelet levels. Platelet levels are therefore predictive for severe organ failure and, already early after the onset of first symptoms, a useful marker for identifying patients at risk for severe disease.
Conclusion: Gender or pre-existing conditions did not influence the clinical presentation in hantavirus infected patients, however severe courses might be predicted for patients with low platelet levels.

O551 Epidemiology and molecular characterization of West Nile virus infection in north-eastern Italy

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Objectives: In Italy, an equine outbreak of West Nile virus (WNV) infection was reported in 1998 in Tuscany region. The virus re-emerged in 2008 with the occurrence of equine and human cases of WNV neuroinvasive infection in Veneto and Emilia Romagna regions. Aim of the study was to report WNV surveillance activity performed in Veneto region in 2008–2009 and to investigate WNV strains circulating in Italy.
Methods: Since September 2008, our Centre performed surveillance of WNV disease by the following diagnostic tests: WNV-RNA detection in plasma and CSF, WNV isolation in Vero cell cultures, detection of WNV IgM and IgG by ELISA followed by PRNT confirmation. A seroepidemiology survey in at risk population resident in Rovigo province was also performed.
Results: Five cases (4 identified retrospectively) of WNV meningoencephalitis and 1 of WNV fever were identified in August-September 2008 and other 6 cases of WNV neuroinvasive disease were identified in August-September 2009; 11 cases were resident in Rovigo province and 1 in the border area between Venice and Rovigo provinces. The estimated incidence of WNV encephalitis in Rovigo province was 2.5 cases per 100,000 population, while WNV seroprevalence was about 0.7%. For the first time in Italy, WNV was isolated from a NAT-positive blood donation done in Rovigo. Whole genome sequence analysis showed the new isolate was phylogenetically related to the Italy-1998 equine isolate and to other recently isolated European strains, with acquisition of new aminoacid mutations, including NS3-Thr249Pro. Molecular modelling

of the effect of this mutation predicted a higher stability of the NS3 protein at high temperatures, such as in avian hosts.
Conclusion: Since 2008, an outbreak of WNV infection, characterised by the occurrence of cases of severe meningoencephalitis, is ongoing in north-eastern Italy. Genome sequencing of WNV isolates and molecular modelling has provided insight into the mechanism of WNV re-emergence in Italy, since the virus has acquired the Thr249Pro change in WNV-NS3 helicase, a trait associated with avian virulence, rapid geographic diffusion, and human outbreaks.

O552 Human babesiosis: an emerging zoonosis also in Italy? Preliminary serological data

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Objective: Aim of this study was to investigate on the seroprevalence of babesioses in people resident/working in areas where some *Babesia* species have been detected in the animals [1–4].
Methods: Blood samples (n = 687) from clinically healthy people (foresters, veterinarian, breeders, residents) living in at risk areas of Northern and Central Italy were collected. Sera obtained were tested for the presence of specific IgG by using the indirect immunofluorescent assay on sale for *B. bovis, B. canis, B. equi* and *B. microti* (opportunely adapted), and the western blot by us recently designed for *B. divergens.*
Results: A total of 21 out of the 240 (8.75%) sera so far analysed proved reactive: 2/32 (6.2%) to *B. bovis,* 2/64 (3.1%) to *B. equi,* and 4/64 (10.4%) to *B. microti.* Sera tested for *B. canis* (n = 32) and *B. divergens* (n = 153) proved negative. No cross-reactions were evidenced.
Conclusions: Our preliminary immunological findings on Italian human population suggest a possible high seroprevalence of babesial infections, greater than to date suspected, that have to be confirmed by the more extensive serological trials programmed. The unexpected high circulation of antibodies to *B. microti* out of the United States confirms the recent report of an autochthonous case of human babesiosis due to *B. microti* from Europe [5]. However, it requires to be studied in depth, by the molecular analysis of the corresponding coagula in some cases available, which may allow not only the conclusive identification of the species involved, but also its genetic relationship with the clustering strains.
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Worldwide dissemination of resistances

O553 First description of carbapenem non-susceptible *K. pneumoniae* isolates from Germany harbouring the OXA-48 carbapenemase

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Objectives: Multidrug resistance in Enterobacteriaceae is of utmost clinical importance since no new antibiotics with activity against gramnegative bacteria will be introduced in the next five years. OXA-48 carbapenemases have been described as an emerging resistance mechanism recently.
Methods: Susceptibility testing was performed by Vitek 2 and Etest. Carbapenemase production was detected by a microbiological assay employing cell-free extracts and parallel testing of the effects of selective β-lactamase inhibitors. In addition a modified Hodge test was performed. PCRs for known carbapenemase gens followed by sequencing were performed. The number of β-lactamases was determined by isoelectric focusing. A pulsed-field gel electrophoresis (PFGE) was performed and Plasmid transfer by transformation was tried.
Results: Four non-copy *K. pneumoniae* isolates from an universital hospital in Freiburg (Southern Germany) were tested because of elevated MICs in Vitek 2. The modified hodge test was positive in all isolates and the micorbiological assay demonstrated a carbapenemase activity which was not inhibited by EDTA, clavulanic acid, cloxacillin or 3-aminophenylboronic acid. Imipenem MICs varied between 1 mg/L and >32 mg/L, meropenem MICs were in the range of 0.38 mg/L til >32 mg/L. PCRs for KPC, VIM, IMP and GES were negative, whereas PCRs and subsequent sequencing showed the presence of OXA-48. Transformation of an OXA-48 harbouring succeeded; no resistance to non-β-lactam antibiotics was co-transferred. PFGE band patterns were related in all four isolates. By isoelectric focusing two β-lactamases with a pI of 5.4 and 7.2, respectively, were found.
Conclusion: This is the first description of OXA-48 in *K. pneumoniae* isolates from Germany. Some strains showed carbapenem MICs in the susceptible range, highlighting the difficulties in detection of this resistance mechanism.

O554 Multidrug-resistant *Klebsiella oxytoca* carrying blaIMP-8 associated to OXY-hyperproduction isolated in an intensive care unit from a community Spanish hospital

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Objective: To study the antimicrobial resistance mechanisms of broad-spectrum cephalosporin-resistant *Klebsiella oxytoca* strains with reduced susceptibility to carbapenems isolated in an intensive care unit from a 200-bed community hospital.
Methods: Nine *K. oxytoca* isolates recovered from clinical (4) and surveillance (5) cultures during a 3-month period were studied. Susceptibility testing was performed by broth microdilution and disc diffusion (CLSI). Phenotypic β-lactamase characterisation included screening and confirmatory test for ESBL, modified Hodge test for suspected carbapenemase production (CLSI), and isoelectrofocusing (IEF). β-lactamase genes were analyzed by PCR and sequencing. The clonal relationship among isolates was determined by PFGE with XbaI and cluster analysis was conducted by using UPGM with tolerance 1%.
Results: All isolates were intermediate or resistant to β-lactams (amoxicillin–clavulanate, piperacillin–tazobactam, cefoxitin, cefotaxime, ceftazidime, cefepime, and aztreonam), quinolones, trimethoprim-sulphamethoxazole, tobramycin and tigecycline. Susceptibility was retained only to gentamicin, amikacin, colistin, and carbapenems (MIC of imipenem: 2 mg/L; MIC range of meropenem: 0.5–1 mg/L; MIC range of ertapenem: 1–2 mg/L). The nine isolates showed a negative double disc test for ESBL detection and a positive modified Hodge test for carbapenemase detection. IEF showed two bands per strain, one with pI slightly higher than 6.5 and another with pI of 8.2. All isolates showed an indistinguishable profile by PFGE (100% similarity). The gene encoding the metallo-β-lactamase IMP-8 was found in all isolates. In addition, all strains presented a G-to-A transition at position 5 of the −10 hexamer of the blaOXY promoter, which has been associated to an increased amount of β-lactamase produced (OXY-hyperproduction), explaining the resistance to aztreonam displayed by all strains.
Conclusion: An outbreak caused by multi-drug resistant *K. oxytoca* in ICU patients from a community hospital was microbiologically characterised. This is the first report on IMP-8-producing *K. oxytoca* in Spain.

O555 Multifocal detection of KPC-producing *Klebsiella pneumoniae* in Italian hospitals

M.M. D'Andrea, T. Giani, G. Brigante, L. Santoriello, G. Ortisi, P. Casella, G. Riccio, G. Gesu, F. Luzzaro, G.M. Rossolini (Siena, Lecco, Savona, Milan, IT)*

Background: Worldwide emergence and spreading of *Klebsiella pneumoniae* carbapenemases (KPCs) is a matter of clinical concern. In Italy, a single KPC-producing *K. pneumoniae* isolate was reported until now (Florence University Hospital, October 2008). Here we report the multifocal detection of KPC-positive nosocomial isolates of *Klebsiella pneumoniae* obtained from inpatients admitted to different Italian hospitals during 2009.
Methods: Antimicrobial susceptibility was evaluated using the VITEK2 system (bioMérieux, Marcy l'Etoile, France) and confirmed by the Etest method (bioMérieux). Synergy tests based on EDTA and boronic acid were performed to screen for different carbapenem resistance mechanisms. Carbapenemase production was first studied by the modified Hodge test and then confirmed by PCR using primers specific for the detection of blaKPC-type alleles. Molecular typing was also performed to assess clonal relationship.
Results: From May to October 2009, KPC-producing *Klebsiella pneumoniae* isolates were detected in 5 Hospitals located in Northern Italy. Isolates showed a multidrug-resistant phenotype, including (in addition to β-lactams), fluoroquinolones, amikacin, tobramycin, and trimethoprim-sulfamethoxazole. MICs of ertapenem, imipenem, and meropenem were $\geqslant$8 mg/L. Susceptibility was retained only for gentamicin, colistin, and tigecycline. Molecular typing showed the occurrence of few clones, although most of strains were clonally related. Epidemiological analysis revealed that two patients had been transferred among hospitals causing inter-hospitals transmission. Intra-hospital diffusion was also observed.
Conclusions: Following the first case reported in 2008, KPC-producing *K. pneumoniae* strains appear to be emerging in Italian hospitals. Due to the ability to rapidly spread, the multifocal detection of these strains is a finding of major concern. Monitoring and epidemiologic surveillance are therefore needed. From a therapeutic point of view, KPC-producing *K. pneumoniae* represent a new challenge for physicians and microbiologists. Based on *in vitro* results, colistin and tigecycline could represent therapeutic options for treating infections caused by these MDR pathogens.

O556 First outbreak of *Klebsiella pneumoniae* producing KPC-2 in France

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Objectives: KPC-producing *K. pneumoniae* (KPC-Kp) are increasingly reported worldwide, mostly in the USA, Israel and Greece, and are associated with higher patient mortality. We report here the first nosocomial dissemination of KPC-Kp isolates mediated by a contaminated duodenoscope.
Methods: KPC-Kp isolates were characterized by standard biochemical methods, by susceptibility testing, by PFGE, plasmid analysis, MLST- and transposon Tn4401-typing. Antibiotic resistance genes were sought by PCR and sequencing.
Results: A 85-year old patient with bladder cancer, (hospital K-Bicetre, France) admitted for severe gastro-intestinal bleeding, underwent endoscopy to stop bleeding. Five days later, he was screened positive for KPC-Kp, resistant to all available antibiotics except gentamicin and colistin. Despite increased awareness and reinforced hygiene precautions, MDR screening of hospitalized patients in the same surgical unit identifed two KPC-Kp(+) patients. Concommitantly, a patient from a neighbouring hospital that underwent endoscopy at the same gastro-enterology ward was diagnosed KPC-Kp positive. These patients had endoscopy on separate days (two weeks apart) but with the same endoscope. Bacterial cultures from this endoscope revealed KPC-Kp. Retrospective analysis of all the patients that had gastroscopy with the same endoscope, identified a Greek patient with KPC-Kp feacal carriage that was directly transferred from the hospital of Chania (Crete, Greece) two months earlier. Since this patient, 17 patients, being mostly from regional hospitals and out-patients, underwent gastroscopy with the same endoscope. Out of 10 patients that could be screened, 6 were KPC-positive and 2 got infected (one bacteriemia and one bilioma) with KPC-Kp.
Conclusion: Although the risk of endoscopy-related infection is low, changes of endoscope reprocessing by replacing glutaraldehyde decontamination bath by automated per-acetic acid washers (to prevent Creutzfeldt-Jacob), may have been deleterious to this endoscope. This report identified he spread of a panresistant enterobacterial isolate in our hospital, and at a regional level in Paris.

O557 First detection in Europe of plasmid-mediated fluoroquinolone resistance qnrD determinant

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Objectives: To investigate the presence of the newly-described plasmid-mediated determinants of quinolone resistance qnrC and qnrD among Enterobacteriaceae isolated in the North-East of Italy.
Materials and Methods: We collected 756 non-replicate Enterobacteriaceae, isolated during 2007 and 2008 in five different microbiology laboratories in the North-East of Italy and selected for being non-susceptible to fluoroquinolones and/or resistant to third-generation cephalosporins, qnrC and qnrD were determined by PCR and sequencing.
Results: No qnrC gene was detected, but five strains (0.66% of the total) presented a qnrD gene. They included four *Proteus mirabilis* and one *M. morganii.*
No ESBL could be found in either *Proteus mirabilis* or *Morganella morganii*. No combination of qnrD with other plasmid-mediated determinant of quinolone resistance was found. All qnrD genes were cloned by heat-shock in *E. coli* DH5α. They were all located in a conjugative plasmid, which was transferred into the *E. coli* J53 host strain and selected with 100 μg/ml of Na-azide and 0.5 μg/ml of ciprofloxacin. The ciprofloxacin resistance of the qnrD-producing clinical isolates carrying the plasmid-mediated determinants widely ranged between $\leqslant$0.06 and 256 mg/L.
Conclusions: QnrD genes have so far been reported only from China, detected for the first time in *Salmonella enterica* and, subsequently, in *E. coli*, and *Klebsiella pneumoniae*. This is the first report of the qnrD gene in Europe, as well as the first detection of this gene in isolates of the tribe Proteeae (namely *P. mirabilis* and *M. morganii)*. This is also the first time that any qnr determinant is found in *M. morganii.*

O558 Occurrence of PMQR determinants and ESBL in clinical Enterobacteriaceae isolates from an Algerian hospital

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Objectives: β-lactams and fluroquinolones are the most commonly prescribed antibiotic classes. The aim of this study was to characterize the mechanisms of resistance to those antibiotics in Enterobacteriaceae clinical isolates from an Algerian hospital.
Methods: Sixty-one Enterobacteriaceae strains collected between January to June 2005 in CHU Mustapha Bacha, Algeria, were included in this study: 33 *K. pneumoniae*, 7 *S. marcescens,* 6 *Enterobacter* spp., 6 *Proteus* spp., 3 *C. kooseri,* 2 *S. typhimurium,* 2 *M. morganii* and 1 *P. rettgeri.* MICs were determined by microdilution broth method. PCR and sequencing were used to screen and identify bla genes (blaTEM, blaSHV, blaOXA, blaCTX-M and plasmid-mediated ampC) and as well as plasmid-mediated quinolone resistance (PMQR) genes (qnrA, qnrB, qnrC, qnrD, qnrS, qepA and aac(6′)Ib-cr). Linkage of blaCTX-M with ISECp1, IS26 and IS903 insertion sequences was investigated by PCR. Biochemical characterization was performed by isoelectrofocusing.

Genetic relatedness among strains was analyzed by pulsed-field gel electrophoresis.
Results: The prevalence of extended-spectrum β-lactamases (ESBL) CTX-M was 45/61 (74%) with 28 CTX-M-15, 16 CTX-M-3 and 1 CTX-M-14 β-lactamases. Other β-lactamase families were also identified, such as CMY-2 (n = 1), TEM-type (n = 60) and SHV-type (n = 35). IEF confirmed strains as ESBL producers. The association of blaCTX-M genes with other bla genes in the same isolate, namely blaTEM-1 or blaSHV-1, was observed. Multidrug resistance was presented especially with aminoglycosides family (72%). PMQR genes were detected only in 4 quinolone non-susceptible isolates: 3 *K. pneumoniae* strains had the aac(6′)-Ib-cr gene and 1 *M. morganii* strain had the recently described QnrD determinant. PFGE analysis revealed a high clonal heterogeneity (>80% similarity) among *K. pneumoniae* strains (23 unique profile types forming 6 clusters), *S. marcescens* (5 unique profiles types distributed by 2 clusters), and 2 *S. typhimurium* strains belonging to the same cluster.
Conclusions: This study revealed the first report of a qnrD gene from an Algerian Hospital. Our work confirms also the geographic spread in Algeria of CTX-M-type β-lactamases, mainly CTX-M-15, and suggests the horizontal transfer of blaCTX-M genes, mediated by mobile elements. Given the emergence of quinolone resistance strains and the dissemination of ESBLs, their continuous spread would have a distressing development.

O559 16S rRNA methylase containing Enterobacteriaceae in the SENTRY Asia-Pacific region frequently harbour plasmid-mediated quinolone resistance and CTX-M types

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Objectives: 16S rRNA methylases and plasmid-mediated quinolone resistance (PMQR) genes have recently emerged as important resistance mechanisms to aminoglycosides and fluoroquinolones respectively. We examined all 16S rRNA methylase containing Enterobacteriaceae from the Asia-Pacific region (2007–2008) for the presence of PMQR and CTX-M-types, which are already known as a world-wide problem.
Methods: Enterobacteriaceae collected from 10 countries as part of the SENTRY Antimicrobial Surveillance Program during 2007–2008 showing elevated aminoglycoside resistance (amikacin MIC ⩾64 mg/L, and gentamicin and tobramycin ⩾16 mg/L) were tested for the 16S rRNA methylase genes (armA, rmtB, and rmtC) by real-time PCR. Isolates positive for 16S rRNA methylase genes were also screened for presence of PMQR genes (qnrA, qnrB, qnrS, aac(6′)Ib-cr, qepA), and CTX-M encoding genes, using real-time PCR.
Results: 16S rRNA encoding genes were detected in 209 of 4,161 isolates from 5 countries: China (107, 6.9%); India (71, 10.5%); Hong Kong (3, 1.5%); Korea (17, 6.1%), Taiwan (11, 5.0%), among 9 species. armA was found in 136 strains and rmtB in 60 strains; each were observed in all 5 countries. rmtC carrying isolates (n = 13) were only from India, observed in 5 species, where previously only described in *Proteus mirabilis*. 91.4% of all 16S rRNA methylase containing isolates also had either PMQR genes: qnr (n = 79, 38%); aac(6′)Ib-cr (n = 66, 32%); qepA (n = 20, 10%), or CTX-M genes (n = 158, 76%). qepA was found in India and China, exclusively with rmtB and predominantly in *Escherichia coli*. Multiple PMQR resistance mechanisms was surprisingly frequent, with 32 (15.3%) isolates containing two PMQR genes, predominantly qnr in combination with aac(6′)Ib-cr. Close to 50% of all 16S rRNA methylase containing isolates also harboured a PMQR gene in combination with CTX-M types.
Conclusion: These results highlight the increasing problem of multiple-drug resistance among clinical isolates in the Asia-Pacific region, and the importance of vigilant surveillance programs to monitor emerging resistance trends.

O560 Molecular characterization of multidrug-resistant strains of *Salmonella infantis* isolated in Italy from human, animals and environment

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Objective: *Salmonella infantis* represents the third serovar isolated in human infections in Europe, with the majority of strains susceptible to drugs. In Italy, since 2004, it is the fourth serovar isolated, and during 2005–2006 multidrug resistant strains emerged in a region of central Italy. In particular strains with R-type ACSSuTKmSxt were isolated in human, environment and food of animal origin.
The aim of this work was to evaluate the clonal origin of multiresistant S. Infantis strains isolated from different sources; in addition with the purpose to analyze the molecular basis of antibiotic resistance, resistance gene cassettes were identified and their localization investigated.
Methods: Seventy-two *S. infantis* strains, isolated between 2002–2008, both susceptible or resistant to antimicrobial drugs, were tested by Pulsed Field Gel Electrophoresis (PFGE) according to Pulse-net protocol. Cluster analysis of PFGE profiles was performed using Bionumerics software. Strains with R-type ACSSuTKmSxt have been tested by PCR for the presence of the following gene cassettes: blaTEM, tetA-B-C-G, sul2, strA-B and class 1 integron. Conjugation experiments have been performed in order to establish the location of resistance genes. Plasmids from parental and transconjugant strains were assigned to incompatibility group by PCR-based replicon typing (PBRT).
Results: Cluster analysis performed on PFGE profiles showed a main cluster (genetic similarity >90%) including 49 strains, of which 27 with R-type ACSSuTKmSxt.
All the 27 strains showed the following resistance genes: blaTEM, tetB, strA-B, sul2, conferring resistance to ampicillin, tetracycline, streptomycin and sulphonamides. In addition they harbour a class 1 integron of 2.2 Kb, including folA, catB3, aadA4, sul1 gene cassettes, which confer resistance to trimethoprim, chloramphenicol, streptomycin/spectinomicyn and sulphonamides. Conjugation experiments showed a unique plasmid harbouring all the resistance genes and belonging to HI1 incompatibility group.
Conclusion: Molecular typing by PFGE and the identification of a plasmid harbouring the resistance gene cassettes demonstrated the circulation of a cluster of S. Infantis, R-type ACSSuTKmSxt, during 2005–2006 in a region of central Italy. The presence of a plasmid conferring multidrug resistance could have facilitated the spread of this clone through the environment, food and human.

O561 Multidrug resistance in *Salmonella* isolates recovered from different food sources in Colombia

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Objectives: *Salmonella enterica* is the most common etiological agent of food borne salmonellosis worldwide. Ciprofloxacin is the antibiotic of choice for the treatment of these infections. Until date, fluoroquinolone resistance remains uncommon in this bacterium. However, the incidence of nalidixic acid resistance is increasing with isolates showing decreased susceptibility to fluoroquinolones. We have screened a large collection of *Salmonella* strains to determine the incidence of multi-drug resistance (MDR) and to further characterize the genotypic and phenotypic mechanisms that contribute to this MDR.
Methods: From 2002–2009, 93 *Salmonella* strains from Colombian foods and exotic animals were obtained from the University of Cordoba (Colombia). The serovar and the susceptibility profile of each strain were determined. Antibiotics representative of 8 different classes were tested and strains that demonstrated resistance to nalidixic acid (NA) were further characterized. PCR was performed to determine the presence of qnr genes. Additionally, efflux activity was evaluated by the EtBr-agar cartwheel method. *Ex vivo* studies were conducted to assess potential differences on the infection and adherence ability of the isolates.

Results: The most predominant serovars in these isolates were: Uganda (n = 19), Anatum (n = 14), Newport (n = 11) and Braenderup (n = 10); although in total 19 different serovars were obtained. Thirteen isolates were resistant to NA (and/or other antibiotics). Four strains showed increased efflux activity when compared with the controls. This was further confirmed by determining the minimum inhibitory concentration of NA in the presence of the efflux pump inhibitor: phenylalanine-arginine-β-napthylamide (PAβN). Resistance to quinolones was confirmed by PCR through the identification of the qnrB19 gene that confers low-level resistance to this class of antibiotics. *Ex vivo* assays were useful to clarify the infection and adherence potential of the isolates.
Conclusion: In this study, a large collection of *Salmonella* isolates obtained from food and exotic animals were resistant to NA and/or other class of antibiotics. These isolates showed different serovars and increased efflux activity along with the presence of the qnrB19 gene. The *ex vivo* assays contributed to clarify the infection and adherence potential of the isolates. These data highlight the importance of intrinsic and acquired mechanisms of MDR in *Salmonella*.

O562 Increasing prevalence and population dynamics of MBL-producing *Pseudomonas aeruginosa* in Russian hospitals

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Objectives: To determine the trends in the prevalence and population genetic structure of MBL-producing *P. aeruginosa* in Russian hospitals over the period of 2002–2007.
Materials and Methods: A total of 1,840 consecutive non-duplicate nosocomial *P. aeruginosa* isolates collected in 48 hospitals of 31 cities of Russia as part of two national surveillance studies in 2002–2004 (n = 1,053) and in 2006–2007 (n = 787) were tested for MBL production using EDTA-double-disk synergy test and PCR assays for blaVIM and blaIMP genes. All MBL-producing isolates were typed by automated fluorescent MLVA; selected isolates of different MLVA clusters were typed by MLST. Structures of class 1 integrons carrying MBL-gene cassettes were analyzed using Taq I restriction endonuclease digestion of PCR fragments generated using outward primers internal to blaVIM or blaIMP genes and primers to 5′ (intI1) and 3′ (qacEdelta1 or tniC) conserved integron sequences. The obtained PCR-RFLP profiles were compared to those of the known integrons.
Results: A total of 48 (4.6%) and 158 (20.1%) isolates collected, respectively, in 2002–2004 and in 2006–2007 were found to produce MBLs. During the first and second study periods MBL producers were identified correspondingly in 3 of 21 and 12 of 26 cities surveyed. By MLVA analysis, all MBL-positive isolates were distributed into two genetic clusters: the predominant one encompassing 23 related MLVA types and corresponding to sequence type (ST) 235 and a minor one comprised of 6 related MLVA types and corresponding to ST234. Isolates of the predominant genetic cluster carried 4 different MBL-integrons whose RFLP profiles matched those of the known integrons harboring blaVIM-2 (GenBank acc. nos. DQ522233 (n = 190), DQ522234 (n = 1), DQ522235 (n = 3)) and blaIMP-1 (DQ522237 (n = 3)). Isolates of the minor genetic cluster carried the blaVIM-2-containing integron (GenBank acc. no. DQ522236; n = 9). VIM-2-producing isolates of the two genetic groups have concomitantly increased in prevalence (ST235: from 4.5% to 18.1%; ST234: from 0.1% to 1.0%) and geographic distribution between the two study periods.
Conclusion: A dramatic increase in the prevalence of MBL-producing *P. aeruginosa* in Russian hospitals between the periods of 2002–2007 to 2006–2007 was noted owing mainly to a clonal spread.

Community-acquired pneumonia

O563 Usefulness of sputum Gram stain and culture for *S. pneumoniae* and *Haemophilus* spp. in the aetiologic diagnosis of community-acquired lower respiratory tract infections and predictive value of serum PCT levels for these bacterial LRTI

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Objectives: To evaluate (1) the usefulness of sputum Gram stain and culture when applied routinely in the etiologic diagnosis of Community-acquired Lower Respiratory Tract Infections (CA-LRTI) in the GRACE study and (2) to correlate sputum culture results with PCT values in serum.
Methods: From 10/2007 through 05/2008, 711 adult patients with LRTI in the community were included during the first winter in a 3 year prospective study in 11 primary care centers in 8 European countries. Sputum and serum specimens were collected before possible antibiotic therapy was started. Sputa were sent to the local laboratory and processed immediately; sera were stored at −70ºC until shipment to the central lab. Quality of sputa was scored according to the number of leucocytes (WBC) and squamous epithelial cells: specimens with ratios of WBC/epithelial cells >1 were defined as good quality sputa. Gram stain and culture were performed according to a standardized protocol. Culture was considered diagnostic when *S. pneumoniae* or *Haemophilus* spp were isolated as a predominant microorganism.
Kryptor based procalcitonin (PCT) assay (BRAHMS) was performed according to the manufacturer's instructions: interpretation of PCT values in relation with bacterial infection was done according to Christ-Crain (Lancet 2004).
Results: Of 711 patients included, 538 (75%) produced sputum: 254 (47.2%) were of good quality. A total of 117/538 (21.7%) of sputa were culture positive: 80 (14.9%) and 37 (6.9%) were positive for *Haemophilus* spp. and for *S. pneumoniae* respectively. 59/80 (73.8%) of *Haemophilus* spp and 25/37 (67.6%) all *S. pneumoniae* positives were from good quality sputa respectively. A predominant morphotype in Gram stain was observed in 53/80 of *Haemophilus* spp and in 33/37 of *S. pneumoniae* positive sputa resulting in sensitivities of 66.3% and 89.2% respectively.
In 69/80 (86.3%) and in 35/37 (94.6%) of *Haemophilus* spp. and *S. pneumoniae* positive sputa respectively, PCT values were <0.1μg/L and 0.1–0.25μg/L indicating respectively absence of bacterial infection or bacterial infection unlikely.
Conclusion: In GRACE, a good quality sputum was obtained from a considerable number of patients presenting with LRTI and culture of these specimens had a good diagnostic yield. Gram stain is more sensitive for the detection of pneumococcal CA-LRTI compared to the detection of *Haemophilus* spp. PCT was found insensitive to predict bacterial infections and guide antibiotic use.

O564 Correlation of the bacterial aetiology with initial procalcitonin levels and duration of antibiotic therapy in lower respiratory tract infections

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Objectives: Procalcitonin (PCT) has been established as a reliable and efficient marker for the differentiation between viral and bacterial lower respiratory tract infections (LRTI) and to guide antibiotic therapy. There is conflicting data about a correlation of higher PCT levels with Gram-negative bacteraemia. In the current analysis we correlate the bacterial aetiology with initial PCT-levels and the duration of antibiotic therapy in LRTI.
Methods: Initial PCT levels of patients hospitalized with LRTIs as part of the Swiss multicenter randomized controlled ProHOSP Study were correlated with the identified bacterial aetiology and total antibiotic duration in survivors. PCT was measured using the highly sensitive

Kryptor® (BRAHMS) assay. Bacteria were identified using standard microbiological cultures from blood and sputum and urinary antigen tests for pneumococcal and legionella (Binax®). Statistical analysis was performed using Mann–Whitney U-Test and Spearman correlation coefficient.
Results: The current analysis includes 388 patients with LRTIs (age 70.5 + 16.7 years) from 2 of the 6 ProHOSP hospitals. *Streptococcus pneumoniae* (45; 23 of those bacteraemic) was the most common known aetiology, followed by legionella (8), and Enterobacteriaceae (5). Other Gram-positive organisms were identified in 12, other Gram-negative organisms in 6 and in 306 patients no causative organisms could be detected. LRTI caused by legionella and Enterobacteriaceae showed similar initial PCT values (median 1.46, $p = 0.31$; and 4.09, $p = 0.80$, respectively) as pneumococcal infections. PCT values were higher for pneumococcal LRTI (median 9.10μg/L) than for other Gram-negative organisms (PCT median 0.30, $p = 0.04$), other Gram-positive organisms (PCT median 0.40, $p = 0.01$) and if no organisms were identified (PCT median 0.25, $p < 0.001$). Total antibiotic treatment duration was guided according to PCT kinetics based on predefined cut-off ranges.
Conclusion: Initial PCT values differed according to the aetiology of LRTIs with more virulent organisms including pneumococci being associated with higher levels. Considerable overlap in mean PCT values precluded prediction of the aetiology based on PCT values alone. However, combining knowledge of initial PCT values and causative organisms might predict the required antibiotic duration. At the meeting, we will present the data of the entire 1359 patients of all 6 ProHOSP hospitals.

O565 Clinical features and outcomes of community-acquired pneumonia due to *Haemophilus influenzae*

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Objective: We sought to determine the clinical features, outcome, and risk factors of community-acquired pneumonia (CAP) caused by *Haemophilus influenzae*.
Methods: Observational analysis of a prospective cohort of nonseverely immunosuppressed adults with CAP requiring hospitalization from 1995 through 2008.
Results: Of 3421 consecutive CAP episodes, 192 (5.6%) were caused by *H. influenzae*. The diagnosis was established with the use of one or more of the following methods: sputum Gram stain and culture (178), blood culture (23), transthoracic needle aspiration specimen culture (5) and pleural fluid culture (1). β-lactamase production was detected in 25 (13%) of *H. influenzae* isolates. Patients with CAP due to *H. influenzae* were older (69.7 vs 65.9 years; $p = 0.016$), more often male (75% vs 68%; $p = 0.033$), had more commonly chronic obstructive pulmonary disease (COPD) (52% vs 26%; $p \leqslant 0.001$), smoking history (67% vs 56%; $p = 0.001$) and were more frequently classified into high-risk pneumonia severity index (PSI) classes (group IV-V) (69% vs 57%; $p = 0.001$) than the remaining patients. They also had received more frequently prior corticosteroid therapy (10% vs 6%; p =.039) and pneumococcal vaccine (<5 years) (24% vs 15%; $p = 0.001$). Previous hospitalization (<1 year) for pneumonia was more common among patients with CAP due to *H. influenzae* (13% vs 7%; $p = 0.002$). Conversely, they were less likely to had pleural effusion (12% vs 18%; $p = 0.049$) and empyema (1% vs 4.3%; $p = 0.028$). No significant differences were found regarding time to clinical stability, length of hospital stay, and ICU admission. Early (0.5% vs 2.3%; p=.1) and 30-day mortality rates (4.7% vs 7.8%; p=.11) did not differ significantly between groups. Multivariate analysis identified COPD (OR = 2.5, 95% CI 1.8–3.5) and high-risk PSI classes (OR = 1.4, 95% CI 1.02–2.17) as independent risk factors for *H. influenzae* pneumonia.
Conclusions: CAP caused by *H. influenzae* occurs mainly in patients with COPD classified into high-risk PSI classes and is associated with significant morbidity and mortality.

O566 Clinical prediction score for community-acquired *Legionella* pneumonia

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Objectives: Legionnaires' disease (LD) is an underdiagnosed community-acquired pneumonia (CAP) with high mortality. Although urinary antigen testing has facilitated the microbiological diagnosis during the acute-phase, only 52 of 99 microbiologically verified LD-cases from a large outbreak in our region in 2005 tested positive. Previous attempts to design a clinical score have yielded conflicting results. The aim of our study was to assess clinical and biochemical predictors of *Legionella* pneumonia using outbreak patients.
Methods: All patients with CAP referred to the regional hospital during a period of five weeks in May/June 2005 were included in the study. A clinical protocol was initiated during an early phase of the outbreak. Clinical and laboratory data on entry from patients with *Legionella* pneumonia and non-*Legionella* pneumonia were compared.
Results: Complete data sets were present for 91 and 90 patients in the LD-group and non-LD group, respectively. In multivariate logistic regression *Legionella* pneumonia was associated independently with high levels of C-reactive protein (OR 1.009, 95% CI 1.005–1.013), presence of muscle pain (OR 2.8, 95% CI 1.4–5.9), low serum sodium concentration (OR 0.91, 95% CI 0.84–0.98), and high body temperature (OR 1.45, 95% CI 1.00–2.11). The continuous variables were dichotomized according to the optimal cut-off value. A simple predictive score was derived by assigning one point for each variable (CRP > 175 mg/L, presence of muscle pain, serum sodium <134 mmol/L, temperature >38.5 centigrades) resulting in a maximum of 4 points. The area under the receiver operator characteristic of the combined score was 0.85 (95% CI 0.79–0.90), which was better than each parameter alone. The median predictive score was higher in the *Legionella* group (2 (IQR 2–3) vs. 1 (IQR 1–2), $p < 0.001$). The sensitivity and specificity of a score of ⩾3 points was 65% and 92%, respectively, with corresponding values of 86% and 66% for a score of ⩾2 points. In the subgroup of LD patients with negative *Legionella* urinary antigen test (n = 45), a score of ⩾3 points and ⩾2 points yielded nearly unaltered sensitivity of 67% and 82%, respectively.
Conclusion: A simple score including four routine clinical and biochemical parameters can be used to predict *Legionella* as aetiological agent of pneumonia, including patients with a negative urinary antigen test. This score will be validated in an upcoming prospective CAP-study.

O567 Influence of antibiotic first-dose timing on clinical outcomes in patients with community-acquired pneumonia

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Objectives: Databased studies have demonstrated the benefits of an early antibiotic treatment in community-acquired pneumonia (CAP) but results from recent experiences have been less consistent. The objective was to study differences in clinical outcomes between those patients who received antibiotics within 4 h (early antibiotic treatment (ET)) of hospital admission and those who received them after 4 h (delayed antibiotic treatment (DT)).
Methods: Prospective study including consecutively all patients diagnosed with CAP and treated with ceftriaxone from July 2006 until-February 2007. Main outcome measures: demographics, pneumonia severity index (PSI), length of hospital stay (LOS), length of antibiotic treatment (LOT), time to clinical stability (TCS) (measured as signs and symptoms resolution) 30-day-mortality and 6-month-mortality. In statistical analysis, "U" Mann–Whitney test for dichotomic variables and Fischer exact test for continuous variables were employed. A value of $p < 0.05$ was considered statistically significant.
Results: total patients: 118. Data comparing early ET versus DT are showed in table1.
Conclusions: Only differences in LOS were observed after comparing ET versus DT. Mean LOS was 2.5 days shorter with antibiotic

administration within 4 hours than with later administration. Timing was not associated with LOT, TCS or short- and long-term mortality.

Mean values	Early treatment (47 patients)	Delayed treatment (71 patients)	p
Age (years)	70.6 (95% CI 66.1–75.1)	71.9 (95% CI 68.1–75.8)	0.523
Male (%)	35 (74.5%)	45 (63.4%)	0.207
PSI class I	3 (64%)	4 (5.6%)	0.900
PSI class II	1 (2.1%)	2 (2.8%)	
PSI class III	7 (14.9%)	14 (19.7%)	
PSI class IV	20 (42.6%)	24 (33.8%)	
PSI class V	16 (34.0%)	27 (38.0%)	
LOS (days)	12.0 (95% CI: 9.9–14.1)	14.5 (95% CI 12.0–17.0)	0.041
LOT (days)	11.3 (95% CI: 10.0–12.6)	11.6 (95% CI 10.2–13.0)	0.978
TCS (days)	11.5 (95% CI: 8.6–14.5)	11.5 (95% CI 9.8–13.1)	0.286
30-day mortality	5 (10.6%)	7 (9.9%)	0.891
6-month-mortality	7 (14.9%)	11 (15.5%)	0.929

O568 FOCUS 2: randomized, double-blinded, multicentre phase 3 study of the efficacy and safety of ceftaroline vs. ceftriaxone in community-acquired bacterial pneumonia

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Objectives: Community-acquired bacterial pneumonia (CABP) is a major cause of morbidity and mortality. *Streptococcus pneumoniae* remains the most common pathogen and there are growing concerns about the rising incidence of resistant or highly virulent pathogens, such as multidrug-resistant *S. pneumoniae* (MDRSP) and community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA). Ceftaroline (CPT), a novel, parenteral, broad-spectrum cephalosporin with bactericidal activity against common Gram-positive organisms, including MDRSP and MRSA, as well as common Gram-negative pathogens, was evaluated for the treatment of CABP (Clinical Trials ID NCT00509106).
Methods: Hospitalized adult patients from 84 centres in 14 countries with moderate to severe CABP (PORT Risk Class III or IV) requiring intravenous therapy received CPT 600 mg q12 h or ceftriaxone (CRO)1 g q24 h for 5 to 7 days (randomized 1:1). Clinical and microbiological responses and adverse events (AEs) were assessed. The primary objective was to determine non-inferiority (pre-specified CI margin of −10%) in clinical cure rate of CPT compared with CRO at the test-of-cure visit (8 to 15 days post-therapy) in the clinically evaluable (CE) and modified intent-to-treat efficacy (MITTE) populations. Secondary objectives included evaluation of clinical cure in the microbiologically evaluable (ME) and microbiological modified intent-to-treat efficacy (mMITTE) populations.

Population	Clinical cure rate		
	Ceftaroline % (n/N)	Ceftriaxone % (n/N)	Difference (95% CI)
CE	82.1 (193/235)	77.2 (166/215)	4.9 (–2.5, 12.5)
MITTE	81.3 (235/289)	75.5 (206/273)	5.9 (–1.0, 12.7)
ME	81.2 (69/85)	75.0 (57/76)	6.2 (–6.7, 19.2)
mMITTE	80.0 (72/90)	75.0 (66/88)	5.0 (–7.4, 17.4)
Streptococcus pneumoniae	83.3 (35/42)	70.0 (28/40)	
Staphylococcus aureus	66.7 (10/15)	56.3 (9/16)	

CE = clinically evaluable; CI = confidence interval; ME = microbiologically evaluable; MITTE = modified intent-to-treat efficacy; mMITTE = microbiological modified intent-to-treat efficacy.

Results: Of 622 treated patients, 315 received CPT and 307 received CRO. Baseline characteristics (MITTE) were comparable between groups (mean age 60.6 vs 62.0 years and male sex 60.6% vs 64.1% for CPT and CRO respectively). Clinical cure rates are shown in the Table. Both CPT and CRO were well tolerated; most common AEs for CPT were diarrhoea (3.8% vs 2.9% for CRO), headache (3.5% vs 1.6%), and insomnia (3.2% vs 2.6%). AEs led to study drug discontinuation in 5.1% and 4.2% of patients treated with CPT and CRO, respectively.
Conclusions: CPT demonstrated non-inferiority to ceftriaxone in treating patients hospitalized with moderate to severe CABP in the CE and MITTE study populations of FOCUS 2. Clinical cure rates in the 2 co-primary populations and in patients with a confirmed bacterial infection were numerically higher for CPT compared with CRO. CPT had high clinical cure rates and was well tolerated, with a safety profile similar to ceftriaxone. CPT has the potential to be an effective, well-tolerated treatment option for CABP.

O569 Pneumococcal bacteraemia serotypes and immunization status in a district general hospital in the United Kingdom

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Objective: Invasive pneumococcal disease (IPD) is a major cause of morbidity and mortality in the United Kingdom. The 23-valent pneumococcal polysaccharide vaccine (PPV) has been recommended for all adults 65 years and over and younger adults with chronic medical conditions or immunosuppression since 2005. The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced into the routine childhood immunization program in September 2006. We aimed to look at pneumococcal bacteraemia serotypes and PCV immunization history in our district general hospital population.
Methods: Patients who had pneumococcal bacteraemia between October 2008 and April 2009 were identified retrospectively. Isolates were sent to the United Kingdom Health Protection Agency reference laboratory for serotyping. Clinical details were acquired via case note review. Immunization status was obtained from general practitioners.
Results: We identified 29 adults with pneumococcal bacteraemia and obtained clinical details for 28. There were 18 patients for whom PPV is currently recommended. Of these seven (47%) had been immunized, of whom two (29%) died; eight had no history of immunization, of whom two (25%) died. For three cases immunization history was not obtained. The total mortality for this group of 18 was 28%. Pneumococcal serotype results were available for 23 patients. Of these, six patients had received PPV, of whom four (66%) acquired infections with serotypes covered by PPV. Of the ten patients who had not received PPV, nine had serotypes covered by PPV. In total, 5 isolates (22%) were not covered by PPV or PCV7, and 14 isolates (61%) were covered by PPV but not PCV7. The total number of isolates covered by PPV was 18 (78%) (Table 1).
Conclusion: In this small study, we observed a high rate of mortality in patients for whom PPV is recommended which was similar in those who had (29%) and had not (25%) received PPV. Uptake of vaccine where recommended was sub-optimal (39%). We observed four cases of apparent failure of the PPV vaccine to protect against IPD with pneumococcal serotypes covered by PPV. This is in keeping with observations that PPV is incompletely protective against IPD. Of the 23 isolates serotyped, 14 (61%) were covered by PPV but not PCV7. As serotypes may spread from the paediatric to the adult population, these may represent serotype-replacement following the introduction of PCV7. Promotion of vaccine uptake and ongoing serotype monitoring is warranted.

Table 1: Immunization status and pneumococcal serotypes

Immunized	Number	Serotypes covered by immunizations			
		Not covered	PPV and PCV7	PPV only	PPV total (%)
Yes	6	2	1	3	4 (66)
No	10	1	1	8	9 (90)
Unknown	7	2	2	3	5 (71)
Total (%)	23	5 (22)	4 (17)	14 (61)	18 (78)

O570 Pneumococcal community-acquired pneumonia, serotype distribution and potential vaccine coverage in the Netherlands

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Objectives: We evaluated distribution of serotypes among adults with pneumococcal Community Acquired Pneumonia (CAP) in the Netherlands in order to determine potential vaccine coverage.

Methods: In a prospective, observational study in 22 Dutch hospitals, patients (⩾18years) admitted to the ER with a clinically suspected CAP were included between January 2008 and March 2009. Recent hospitalization (<2 wks), stay in long-term care facilities, known bronchial obstruction or a history of post-obstructive pneumonia, primary or metastatic lung cancer, AIDS/ PCP/ TBC and unconsciousness were exclusion criteria. History taking, physical examination, biochemical and hematological blood testing, blood- and sputum cultures and BINAX pneumococcal urinary antigen testing were performed in all patients. All pneumococcal bacteremia isolates were serotyped (at the Netherlands Reference Laboratory Amsterdam). Pneumococcal CAP was defined on the presence of clinical criteria for CAP, radiographic confirmation by a radiologist and isolation of *S. pneumoniae* from blood or sputum (if dominant flora) or positive antigen testing in urine.
Results: Of 1631 included patients, 1115 had radiographically confirmed CAP and 210 (18.8%) had pneumococcal CAP, diagnosed on isolation of *S. pneumoniae* from blood culture (n = 82, 7.3% of all bloodcultures), sputum culture (n = 24), or positive urinary antigen test (n = 151). 75 bacteremia isolates were serotyped. Serotypes 14 (n = 9), 1, 7F, 19A and 22F (n = 8) were most common. Potential vaccine coverage was 28% for the 7-valent, 67% for the 13-valent pneumococcal conjugate vaccine (PCV) and 93% for 23-valent polysaccharide vaccine (PS).
Conclusion: Based on bacteremia isolates vaccine coverage would be 67% with 13-valent pneumococcal conjugate vaccine that is currently investigated in a double-blind placebo-controlled trial in the Netherlands.

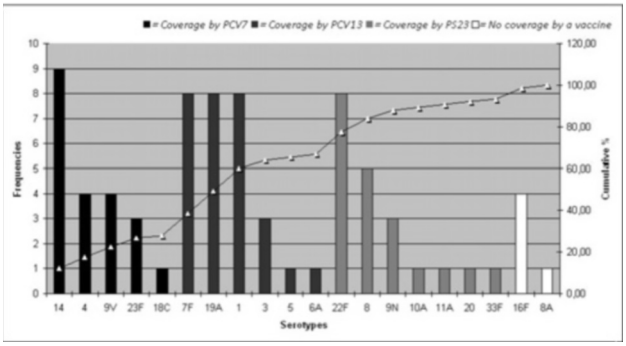

Figure 1. Serotype distribution and potential vaccine coverage.

O571 Serotype-specific mortality risk among patients with invasive pneumococcal disease: Swedish population-based study

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Objectives: To investigate pneumococcal serotype-specific mortality risk correlated to the serotype invasive disease potential among patients with invasive pneumococcal disease (IPD).
Methods: In Sweden it is mandatory to report IPD and isolates are collected for serotyping and molecular typing. Of 4195 episodes of IPD among adults reported between Jan 2007 to Oct 2009 a total of 3692 isolates were serotyped (88%). A questionnaire study was performed to obtain clinical and socio-demographic information about patients with serotyped IPD [obtained for 1657/3692 patients (45%)]. In a preliminary analysis of the 1657 patients we assessed the absolute serotype-specific 30-day mortality risks and relative risks using regression modeling on group level according to previously defined invasive disease potential [high (serotypes 1 and 7), medium (serotypes 4, 9, 14 and 18) and low (serotypes 3, 6A, 6B, 8, 15, 19, 33 and 38) and for individual serotypes. Risk estimates were adjusted for potential confounders.
Results: Pneumococcal serotype prevalence ranged from 0.05% for serotype 5 to 13.4% for serotype 14 and was stable over the observed period. Absolute mortality risk was highest for serotypes with low invasive disease potential (12.8%) followed by serotypes with medium- (8.6%) and high (4.9%) invasive disease potential. Serotypes with low invasive disease potential were associated with increased mortality risk in unadjusted but not adjusted analyses [relative risks 2.62 (95% CI 1.38–5.00) and 1.61 (95% CI 0.84–3.09) respectively] and a test for trend according to invasive disease potential was significant in unadjusted (p-value: 0.001) but not adjusted (p-value: 0.10) analyses. Patients infected with serotypes of lower invasive disease potential were older, more likely to be diagnosed with meningitis, suffer from chronic disease and immunosuppression (all p-values <0.003). Serotype 3, 6B, 11A, 14, 18C, 19F and 23F were associated with increased mortality risk in crude analyses but only serotype 6B and 11A were associated with increased mortality risk in adjusted analyses.
Conclusion: In this large population-based study host risk factors appeared to be the strongest determinants of both risk of infection with serotypes according to invasive disease potential as well as mortality outcome.

O572 Role of atypical pathogens in hospitalized adults with community-acquired pneumonia

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Background: CAP can be caused by a broad spectrum of bacterial pathogens. Appropriate antimicrobial therapy is based on knowledge of common causative agents. Incidence of atypical causative agents of CAP may interfere in treatment choice.
Objectives: To investigate the frequency of atypical pathogens in hospitalized adults with CAP in Smolensk region.
Methods: A prospective study on adult patients with CAP in various Smolensk city hospitals was performed in 2007. Pretreatment respiratory specimens were obtained for routine culture and DNA based polymerase chain reaction (PCR) for the detection of *Legionella pneumophila* (L.p.), *Mycoplasma pneumoniae* (M.p.) and *Chlamydophila pneumoniae* (C.p.). Acute phase and reconvalescent serum samples were collected for the detection of specific IgM and IgG antibodies of L.p., M.p., C.p. by ELISA in accordance with manufacturer's manual [1]. Multiple logistic regression analysis was used to identify independent risk factors (age, CAP severity, presence of complications, significant comorbidities, etc.) of infection with atypical pathogens.
Results: Overall, 295 patients aged from 18 to 87 years, mean age 43.0±19.9 years, 80.7% males, were enrolled. PCR was positive in 57/295 (19.3%) of cases: M.p. – 47/295 (14.9%), C.p. – 5/295 (1.7%), L.p. – 5/295 (1.7%) and M.p. with C.p. – 3/295 (1%). Causative agents were revealed by routine culture in 78/295 (26.4%) of cases. Both PCR and serological tests were performed in 112/295 (38%) of patients. The results coincided in 44/112 (39.3%) of cases; 24/26 (92.3%) of PCR positive cases were confirmed by serologic tests, serology yielded positive results for 18/109 (16.6%), 29/110 (26.4%) and 50/91 (54.9%) PCR negative L.p., C.p. and M.p. samples. Absence of complications and serious comorbidities were only independent variables predicting the high risk of M.p. and M.p. + C.p. infection.
Conclusions: Atypical bacteria (especially M.p.) are rather common causative agents of CAP in hospitalized adults in Smolensk region. The risk of M.p. and/or C.p. infection is increased in uncomplicated CAP cases and patients without serious concomitant diseases. ELISA doesn't seem to be a reliable test for identification of atypical bacterial pathogens.
Reference(s)
[1] Sero MP™ IgM, Sero MP™ IgG, Sero CP™ IgM and Sero CP™ IgG, Savyon Diagnostics; *Legionella pneumophila* Serogroup 1 ELISA IgM and IgG, Vircell, s.1.